Translational Profiling of Chronic Lymphocytic Leukaemia

by

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B cell chronic lymphocytic leukaemia (B-CLL) is the most common form of adult leukaemia in the western world, characterised by the slow accumulation of small mature B lymphocytes in the circulating blood. Disease progression is prolonged and patients may survive for more than 20 years, however it ultimately remains incurable with conventional chemotherapy.

Regulation of gene expression is a complex process and exerted at multiple steps in the expression pathway, including translation. Translational regulation has not previously been studied in B-CLL yet may be important as there are numerous examples of translation de-regulation in tumorigenesis. Results obtained suggest that a reduced level of protein synthesis was occurring in B-CLL cells. Furthermore, cDNA microarray analysis was performed providing a gene expression profile at the level of translation, which correlates with the biology of the disease.

Internal ribosome entry could be a possible mechanism of up-regulation utilised, and evidence is provided to suggest this may be the case. Potential IRES activity was observed for three genes, found to be translationally up-regulated in B-CLL cells by the cDNA microarray analysis, under normal and serum starvation conditions.

The microarray data provides some potential new targets for treatment. In particular, the up-regulation of the angiotensin II type 1 receptor may influence survival of B-CLL cells. Initial evidence suggests that its ligand angiotensin II could influence B-CLL cell survival.

Median survival of B-CLL patients is 10 years, however disease progression is variable; some patients will survive for many years while others have a more aggressive form and die within a couple of years. Progression has been observed to correlate with the mutational status of the immunoglobulin heavy chain variable (I_gV_H) genes. Differences in polysome association for the two subtypes were determined, using the microarray data obtained, identifying a number of genes that may be significant to the biology of the disease.

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Abbreviations

4E-BP	eIF4E-binding protein
aa-UTP	aminoacyl-deoxyuridine 5'-triphosphate
ANG II	Angiotensin II
APAF-1	Apoptotic protease activating factor 1
ATP	adenosine 5'-triphosphate
BCR	B cell receptor
BCL-2	B-cell leukaemia gene 2
B-CLL	B-cell chronic lymphocytic leukaemia
Bp	base pair
BSA	bovine serum albumin
CDK	Cyclin-dependent kinase
cDNA	complementary deoxyribonucleic acid
CIAP	calf intestinal alkaline phosphatase
CTP	cytidine 5'-triphosphate
CXCR4	Chemokine (C-X-C motif) receptor 4
dATP	deoxyadenosine 5'-triphosphate
dCTP	deoxycytidine 5'-triphosphate
dGTP	deoxyguanosine 5'-triphosphate
DMEM	Dubecco's modified eagle medium
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleic acid
DTT	dithiothreitol
dTTP	deoxythymidine 5'-triphosphate
EBV	Epstein Barr Virus
E. coli	*Escherichia coli*
ECM	extracellular matrix
EDTA	Diaminoethanetetra-acetic acid
eIF	eukaryotic initiation factor
eEF	eukaryotic elongation factor
eRF	Eukaryotic release factor

FACs	Flow cytometric analysis
FADD	fas-associated death domain
FCS	Foetal calf serum
GDP	guanosine diphosphate
GTP	guanosine 5'-triphosphate
Ig	immunoglobulin
I_gV_H	immunoglobulin heavy chain variable
IFNγ	Interferon gamma
IRES	internal ribosome entry site
ITAM	immunoreceptor tyrosine-based activation motif
Kb	kilobase
KDa	kilodalton
LB	Luria-Bertani broth
Leu1	deleted in leukemia 1
Leu2	deleted in leukemia 2
MAPK	mitogen-activated protein kinase
MDM2	mouse double minute 2
Met-$tRNA_i$	initiator methionyl tRNA
Mnk	MAP kinase interacting kinase/MAP kinase integrating kinase
mRNA	messenger ribonucleic acid
nt	nucleotide
ORF	open reading frame
PABP	Poly(A) binding protein
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffer saline
PCR	polymerase chain reaction
PLL	prolymphocytic leukaemia
PTK	protein tyrosine kinase
Rb	Retinoblastoma protein
RNA	ribonucleic acid
RNase	ribonucleic acid hydrolase
RPMI	Rose Park Memorial Institute

RT-PCR	reverse transcriptase-polymerase chain reaction
SDF-1	Stromal derived factor 1
SDS	sodium dodecyl sulphate
SV40	simian virus 40
Taq	Thermus aquaticus
TBE	Tris-borate EDTA
TE	Tris-EDTA
TOP	terminal oligopyrimidine tract
TRAIL	TNF-related apoptosis inducing ligand
tRNA	transfer ribonucleic acid
UTR	untranslated region
UTP	uridine 5'-triphosphate
UV	ultraviolet
XIAP	X-linked inhibitor of apoptosis
ZAP70	Zeta-chain (TCR) associated protein kinase 70kDa

Chapter 1

Introduction

1.1 General properties of chronic lymphocytic leukaemia

1.1.1 Overview

B cell chronic lymphocytic leukaemia (B-CLL) is the most common adult leukaemia in the Western world and is characterised by the slow accumulation of small mature B lymphocytes in the circulating blood. The leukaemic cells are not rapidly proliferating but quiescent and thought to grow slower than normal B lymphocytes, with accumulation due to a failure of the B-CLL cells to die (Caligaris-Cappio and Hamblin, 1999). B-CLL is a prolonged disease with patients sometimes surviving for more that 20 years. However, it remains ultimately incurable with current treatments.

1.1.2 Clinical properties

B-CLL is the most common form of adult leukaemia in the Western world, with an incidence rate of about 3 in 100,000 per year (Eisner et al., 2003). However, the true incidence rate is not known, as many patients display no symptoms so the disease goes undiagnosed. It is a leukaemia that mainly affects the elderly, with the average age of diagnosis being 60 and cases under the age of 50 rare. Between 1996 and 2000 in the USA there were 20.6 in 100,000 new cases for those over 65 years, while in those under 65 years, there was only an incidence rate of 1.3 in 100,000 (Eisner et al., 2003). Incidence is also more common in males than females, with a ratio of roughly 2:1 (Eisner et al., 2003). No cause has been determined for B-CLL. There is no evidence for correlation with an environmental risk factor such as radiation or chemical exposure. However genetic factors may affect susceptibility as close relatives of B-CLL patients have been observed to have an increased risk of developing B-CLL or related conditions (Redaelli et al., 2004).

Median survival for B-CLL is 10 years (Dreger and Montserrat, 2002), however progression is variable. Some patients survive for many years, whilst others have a more aggressive form where survival may only be 1 or 2 years. There are two systems that are used for staging B-CLL; the Rai system (1975) and Binet system (1981), which correlate clinical findings with survival times and are used to determine treatment more effectively. Other prognostic factors can also aid in prescribing the best treatment, for example the

mutational status of the immunoglobulin heavy chain variable (I_gV_H) genes has been observed to correlate with disease progression (Damle et al., 1999; Hamblin et al., 1999). (Discussed in further detail in section 1.3). B-CLL can transform into more aggressive forms of leukaemia. A Richter transformation occurs in about 3-5% of B-CLL patients and involves development of a lymphoma in a patient who already has B-CLL. It is much more aggressive than B-CLL and survival may only be months. Transformation into the prolymphocytic form (PLL) occurs in about 10% of B-CLL patients and again prognosis is poor. The lymphocytes in PLL are larger than B-CLL lymphocytes, and extensive splenomegaly and bone marrow infiltration are observed. It is also common for B-CLL patients to develop autoimmune disease, with the development of autoantibodies usually against haematopoietic antigens such as those found on red blood cells or platelets (Kipps, 2003).

No cure is currently available for B-CLL, however treatments that have been developed can prolong survival for many years. The treatment prescribed depends upon the stage of disease and prognostic factors, and at a very early stage of disease patients may not require chemotherapy at all, until the disease has progressed. The drug treatments available include glucocorticoids (prednisone), alkylating agents (chlorambucil and cyclophosphamide) and purine analogs (fludarabine, cladribine and pentostatin). Fludarabine is the most effective drug available at present, however patients will ultimately become resistant to the drugs. Some new biological therapies now available, alemtuzumab (a monoclonal antibody against CD52) and rituximab (a monoclonal antibody against CD20) may improve survival.

1.1.3 General properties of B-CLL cells

B-CLL cells are small B lymphocytes which express most cell surface markers found on mature B-cells localised in the mantle zone (MZ) of secondary lymphoid follicles (Caligaris-Cappio and Janossy, 1985). However, they are characterised by the expression of CD19, CD5 and CD23, and low expression of surface immunoglobulin (usually IgM or IgD) (Caligaris-Cappio and Hamblin, 1999). These characteristics allow B-CLL cells to be distinguished from other types of B-lymphocytes, including other leukaemias and lymphomas (Harris et al., 1994).

The origin of B-CLL cells is unknown. Until recently, it was believed that they were derived from malignant transformation of CD5+ B cells, found in the mantle zone of

secondary lymphoid follicles, like B-CLL cells (Caligaris-Cappio, 1996). They are also similar to B-CLL cells in the co-expression of CD5 and low surface immunoglobulin. However, recent microarray analysis has determined that the gene expression profile of B-CLL cells is more closely related to that of memory B cells (Klein et al., 2001).

B-CLL cells are not rapidly proliferating but are quiescent and found in the G0 phase of the cell cycle (Andreeff et al., 1980; Caligaris-Cappio and Hamblin, 1999). Accumulation of leukaemic cells is thought to occur through defective apoptosis (Caligaris-Cappio and Hamblin, 1999). B-CLL cells are resistant to apoptosis *in vivo* (Osorio et al., 1998) but rapidly apoptose *in vitro* (Collins et al., 1989; MacFarlane et al., 2002) and are therefore thought to require signals from the *in vivo* microenvironment for their survival.

1.1.4 Chromosome abnormalities

A number of chromosome abnormalities have been observed in B-CLL, although no single common defect has been found (Caligaris-Cappio, 2000). Translocations are rare but a number of chromosome deletions have been observed. The most common of these involves a region of chr13q14 that is telomeric to the retinoblastoma gene RB-1 and is deleted in more than 50% of B-CLL cases and has been observed to be associated with a less aggressive form of the disease (Oscier et al., 1997). It is possible that a tumour suppressor gene may be located in this region. A number of potential genes that could fulfil this role have been cloned from the region, including Leu1 (deleted in leukemia 1) and Leu2 (deleted in leukemia 2) (Corcoran et al., 1998), although no functional role has been determined to date. Recently two microRNAs, miR15 and miR16, have also been found in the region (Calin et al., 2002). MicroRNAs represent a new class of translational regulators so their deletion may have a functional significance (microRNAs are discussed further in section 1.3.8). Deletions at 17q13 and 11q22-q23 have also been observed in B-CLL and are associated with a more aggressive disease (Gaidano et al., 1991; Lens et al., 1997; Dohner et al., 1997). 17q13 deletions are associated with loss of p53 (Gaidano et al., 1991; Lens et al., 1997; Dohner et al., 1997), whilst 11q22-q23 contains the gene for ATM, which may be involved in B-CLL development (Bullrich et al., 1999). Trisomy 12 has also been observed in B-CLL patients and is associated with atypical cell morphology and atypical immunophenotype (Bigoni et al., 1997; Su'ut et al., 1998).

1.2 Biology of B-CLL

1.2.1 Cell cycle status of B-CLL cells

As mentioned previously, circulating B-CLL cells have been observed to be quiescent and found in the G0/G1 phase of the cell cycle (Andreeff et al., 1980; Caligaris-Cappio and Hamblin, 1999). Cell cycle progression is regulated by interactions between cyclins, cyclin dependent kinases (CDK) and CDK inhibitors (Pavletich, 1999). The D type cyclins (D1, D2 and D3) and Cyclin E are important for the G1/S transition of the cell cycle. They bind to and activate specific CDKs; D cyclins bind to CDK4 and CDK6 and cyclin E to CDK2, which then subsequently phosphorylate retinoblastoma protein (Rb). Phosphorylation leads to the release of the transcription factor E2F and cell cycle progression into S phase (Weinberg, 1995; Muller and Helin, 2000) (Figure 1.1). Differential expression of the D cyclins and cyclin E has been observed in B-CLL cells, which may affect cell cycle progression. Cyclin D2 has been observed to be overexpressed at the mRNA and protein level (Delmer et al., 1995; Woloweic et al., 2001). Contrasting observations have been found for cyclin D3 with some studies finding it to be expressed in B-CLL cells (Woloweic et al., 2001; Suzuki et al., 1999), whilst it was not detectable in another (Delmer et al., 1995). Cyclin E levels have also been observed to be higher in B-CLL cells than normal peripheral blood B cells (Woloweic et al., 1995; Decker et al., 2004). Expression of G1 cell cycle regulatory factors suggested that B-CLL cells may not be quiescent but blocked in early G1 phase (Woloweic et al., 2001).

The CDK inhibitor p27 is an important regulator of G1/S cell cycle transition. It binds to and inhibits the activities of cyclin D-CDK4/6 and cyclin E-cdk2 complexes preventing cell cycle progression (Firpo et al., 1994) (Figure 1.1). In normal cells p27 is highly expressed during the G0/G1 phase of the cell cycle, then downregulated when progression to S phase occurs (Coats et al., 1996; Toyoshima and Hunter, 1994). In B-CLL cells p27 has been observed to be overexpressed and may contribute to a G1 cell cycle block (Vrhovac et al., 1998), although expression was found to be variable, with higher expression in patients with poorer prognosis.

Although circulating B-CLL cells are found to be quiescent analysis of telomere length and telomerase activity revealed that the cells had in fact undergone a considerable number of divisions (Bechter et al., 1998; Damle et al., 2004), and have been observed to have shorter

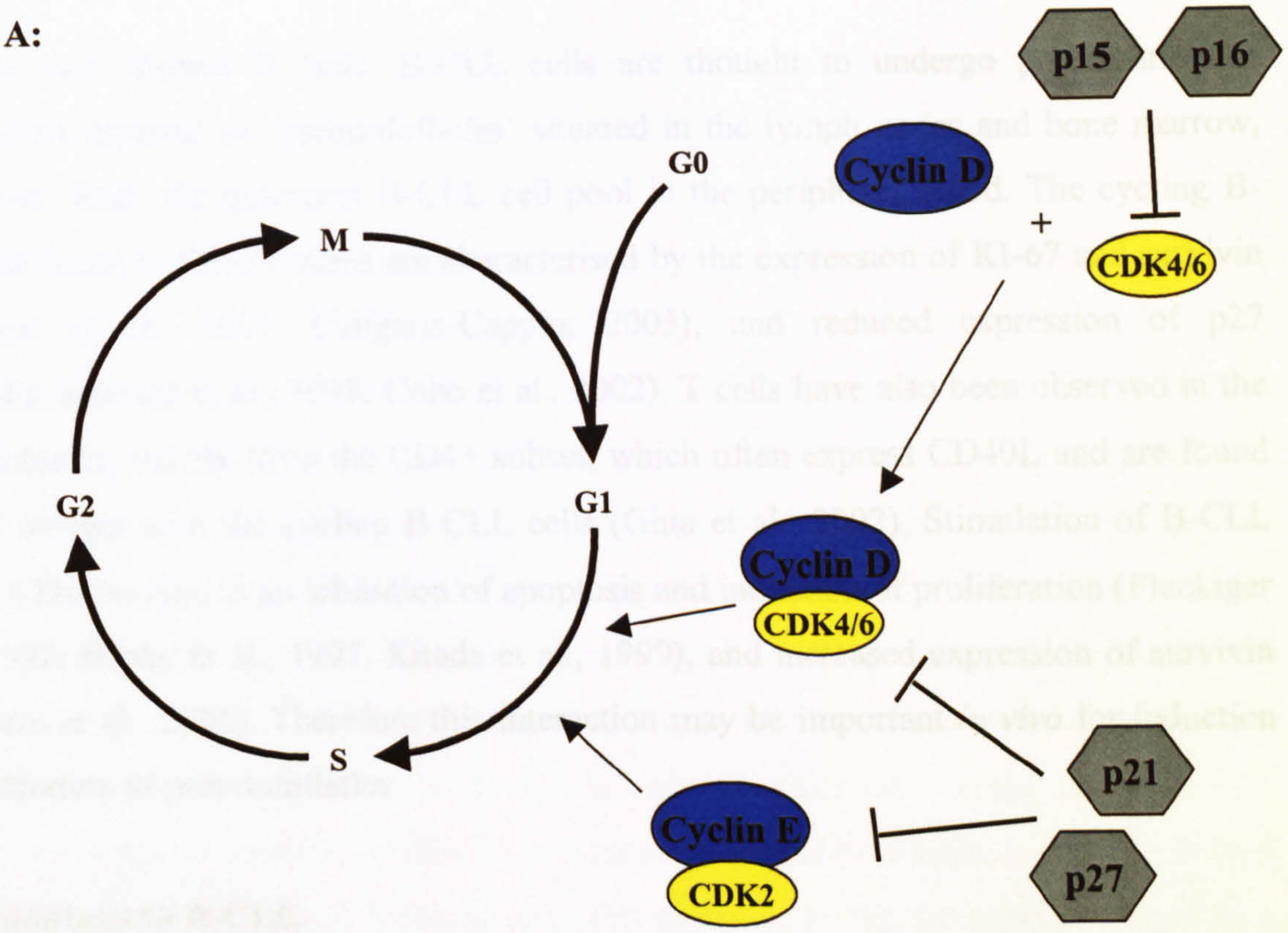


Figure 1.1 Control of G1/S transition of the cell cycle. The Cyclin D/CDK4/6 and cyclin E/CDK2 complexes act during the G1/S transition. This activity can be inhibited by a number of CDK inhibitors (A). The CDKs phosphorylate Rb leading to the release of the transcription factor E2-F, which promotes cell cycle progression to S phase (B).

telomeres than normal B cells. B-CLL cells are thought to undergo proliferation in 'proliferation centres' or 'pseudofollicles' situated in the lymph nodes and bone marrow, which then 'feed' the quiescent B-CLL cell pool in the peripheral blood. The cycling B-CLL cells found in these centres are characterised by the expression of KI-67 and survivin (Granziero et al., 2001; Caligaris-Cappio, 2003), and reduced expression of p27 (Qintanilla-Marinez et al., 1998; Cobo et al., 2002). T cells have also been observed in the pseudofollicles, mostly from the CD4+ subset, which often express CD40L and are found in close contact with the cycling B-CLL cells (Ghia et al., 2002). Stimulation of B-CLL cells by CD40 results in an inhibition of apoptosis and induction of proliferation (Fluckiger et al., 1992; Buske et al., 1997; Kitada et al., 1999), and increased expression of survivin (Granziero et al., 2001). Therefore this interaction may be important *in vivo* for induction of proliferation in pseudofollicles.

1.2.2 Apoptosis in B-CLL

Apoptosis is an important physiological process that allows the removal of unwanted cells in a controlled manner. It is of particular importance in the prevention of tumorigenesis as its failure leads to the accumulation of deregulated cells that may form tumours. Apoptosis is induced by a range of factors, either by activation of cell surface receptors or intracellular activation involving the mitochondria. Activation of cell surface receptors by their ligands, such as Fas/CD95 by Fas ligand, leads to formation of a multi-protein complex at the receptor termed the death-inducing signalling complex (DISC) (Kischkel et al., 1995; Medema et al., 1997). In Fas signalling stimulation of the receptor leads to trimerisation and binding of the adaptor protein Fas-associated death domain (FADD). Procaspase 8 is then recruited to the complex where it becomes activated and initiates a protease cascade, involving other caspases, the effector molecules of apoptosis. They are termed caspases as they have a cysteine residue at their active site and cleave their substrates at aspartate. They are synthesised as inactive zymogens and only become active upon proteolytic cleavage. Caspase 8 is activated by self cleavage when multiple molecules are brought into close proximity at death receptors. Intracellular signals which activate apoptosis, such as DNA damage, cause release of cytochrome c from the mitochondria, Cytochrome c then associates with apoptotic protease activating factor (Apaf-1) and procaspase 9 to form the 'apoptosome' (Cain et al., 1999). Procaspase 9 molecules then activate each other by proteolytic cleavage and initiate a caspase cascade. Both pathways

lead to the activation of effector caspases, which cleave other protein targets in the cell at aspartate, bringing about the execution of apoptosis (Figure 1.2).

There are a number of factors identified that may contribute to the apoptotic resistant phenotype observed for B-CLL cells. Bcl2 has been consistently observed to be overexpressed at the mRNA and protein level in B-CLL cells (Hanada et al., 1993; Robertson et al., 1996). It is an anti-apoptotic protein that is the founding member of a family of apoptotic regulatory proteins, discovered as a chromosomal translocation in human B cell lymphomas (Vaux et al., 1988). The family is characterised by the presence of at least one Bcl2 homology (BH) domain in the protein. Some of the other family members, such as Bcl-XL and Mcl-1, also have anti-apoptotic activity whilst the rest are pro-apoptotic, for example Bax and Bad. The relative expression of the pro- and anti-apoptotic proteins determines whether apoptosis is promoted or inhibited, and act, at least in part by forming homo and heterodimers (Oltvai et al., 1993). In addition, it has been suggested that Bcl-2 proteins may exert their regulation on apoptosis by controlling cytochrome c release from mitochondria (Shimizu et al., 1999; Kluck et al., 1997; Yang et al., 1997). It has not been proven whether overexpression of Bcl2 in B-CLL cells has an essential role in the biology of the disease. However a higher Bcl2:Bax ratio has been shown to be associated with a more aggressive phenotype (Pepper et al., 1996; Pepper et al., 1997; Pepper et al., 1999). Another anti-apoptotic family member, Mcl-1 has been observed to be associated with failure to achieve complete remission after chemotherapy (Kitada et al., 1998).

p53 is a transcription factor that regulates cellular responses to DNA damage (Lane, 1992; Ko and Prives, 1996; Levine, 1997). Upon detection of DNA damage, p53 is activated and induces changes in gene expression, which leads to cell cycle arrest at the G1 checkpoint or induction of apoptosis, the response depending upon cell status and extent of DNA damage. The loss of p53 function is the most frequent abnormality observed in cancer (Bartek et al., 1991; Hollstein et al., 1991) as it leads to a loss of response to DNA damage. p53 is activated by the protein ATM (ataxia telangiectasia-mutated), which integrates cellular responses to DNA damage (Kastan and Lim, 2000) and has also been observed to be mutated or deleted in cases of B-CLL (Bullrich et al., 1999; Stankovic et al., 1999; Pettit et al., 2001; Stankovic et al., 2002), thus resulting in impaired p53 response. p53 has been observed to be mutated or deleted in 10-15% of B-CLL cases (Wattel et al., 1994; Dohner

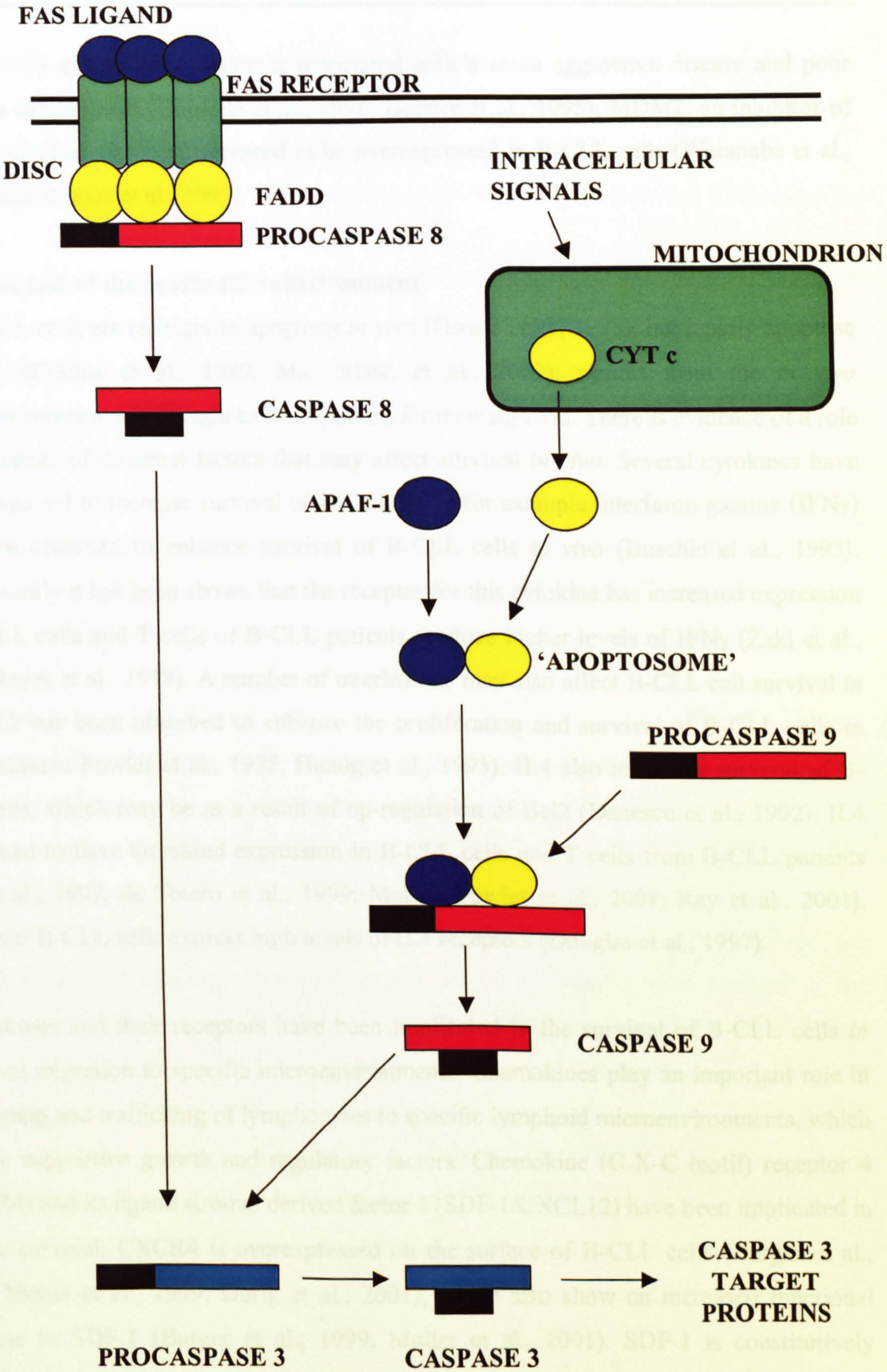


Figure 1.2 The apoptotic pathway. An overview of the apoptotic pathway for Fas-dependent and non-receptor-mediated apoptosis.

et al., 1995) and its dysfunction is associated with a more aggressive disease and poor response to treatment (Cordone et al., 1998; Dohner et al., 1995). MDM2, an inhibitor of p53 protein, has also been reported to be overexpressed in B-CLL cells (Watanabe et al., 1994; Bues-Ramos et al., 1995).

1.2.3 The role of the *in vivo* microenvironment

As B-CLL cells are resistant to apoptosis *in vivo* (Osorio et al., 1998), but rapidly apoptose *in vitro* (Collins et al., 1989; MacFarlane et al., 2002), signals from the *in vivo* microenvironment are thought to be important for their survival. There is evidence of a role for a number of different factors that may affect survival *in vivo*. Several cytokines have been observed to increase survival of B-CLL cells, for example interferon gamma (IFNγ) has been observed to enhance survival of B-CLL cells *in vivo* (Buschle et al., 1993). Subsequently it has been shown that the receptor for this cytokine has increased expression in B-CLL cells and T cells of B-CLL patients produce higher levels of IFNγ (Zaki et al., 1998; Reyes et al., 1998). A number of interleukins may also affect B-CLL cell survival *in vivo*. IL2 has been observed to enhance the proliferation and survival of B-CLL cells *in vitro* (Mainou-Fowler et al., 1995; Huang et al., 1993). IL4 also increased survival of B-CLL cells, which may be as a result of up-regulation of Bcl2 (Danescu et al., 1992). IL4 was found to have increased expression in B-CLL cells and T cells from B-CLL patients (Mu et al., 1997; de Totero et al., 1999; Mainou-Fowler et al., 2001; Kay et al., 2001). Moreover B-CLL cells express high levels of IL4 receptors (Douglas et al., 1997).

Chemokines and their receptors have been implicated in the survival of B-CLL cells *in vivo*, and migration to specific microenvironments. Chemokines play an important role in the homing and trafficking of lymphocytes to specific lymphoid microenvironments, which provide supportive growth and regulatory factors. Chemokine (C-X-C motif) receptor 4 (CXCR4) and its ligand stromal derived factor 1 (SDF-1/CXCL12) have been implicated in B-CLL survival. CXCR4 is overexpressed on the surface of B-CLL cells (Burger et al., 1999; Mohle et al., 1999; Durig et al., 2001), which also show an increased functional response to SDF-1 (Burger et al., 1999; Muller et al., 2001). SDF-1 is constitutively expressed by bone marrow stromal cells (Nagasawa et al., 1994; Bleul et al., 1996) and it was believed that CXCR4 may play a role in B-CLL migration to the bone marrow, especially as it was shown that B-CLL cells could be rescued from apoptosis by contact with bone marrow stromal cells (Lagneaux et al., 1998). However, blood from B-CLL

patients has been found to contain cells that can differentiate into adherent 'nurse like' cells and also expressed SDF-1. These cells have been observed to protect B-CLL cells from apoptosis, through a CXCR4 dependent mechanism (Burger et al., 2000; Tsukada et al., 2002). Therefore, an interaction with the 'nurse like' cells could counteract a migration to bone marrow cells (Barretina et al., 2003).

Adhesion molecules such as integrins could also affect B-CLL migration and survival. These molecules have diverse roles in mediating interactions between cells, and between cells and the extracellular matrix (ECM), which have important roles for control of B cell development and function. Integrins are heterodimeric glycoproteins consisting of noncovalently associated α and ß chains. B-CLL cells consistently express the ß1 and ß2 integrins, plus variable amounts of α3, α4 and α5 chains (Vincent et al., 1996). The ß1 and ß2 integrins have been observed to mediate interactions of B-CLL cells with bone marrow stromal cells and inhibit spontaneous apoptosis *in vitro* (Lagneaux et al., 1998).

1.2.4 BCR signalling in B-CLL cells

The B cell receptor (BCR) is a key signalling molecule in B cells that regulates a number of cellular processes including proliferation, differentiation and apoptosis. It consists of a disulphide bonded complex of heavy and light chain immunoglobulin (Ig) chains, the antigen binding subunit, and a heterodimer of CD79a and CD79b, the signalling subunit. The BCR is unusual in having no unique ligand; each B cell has developed a BCR that can recognise a particular antigen through genetic recombination and somatic mutation of the immunoglobulin genes during B cell development. Upon binding of antigen to the BCR a tyrosine phosphorylation cascade is initiated. Stimulation of BCR leads to phosphorylation of tyrosine residues in CD79a and CD79b, by the protein tyrosine kinase (PTK) Lyn, present in the immune receptor tyrosine based motifs (ITAMs) (Nagai et al., 1995; Johnson et al., 1995). Another PTK, Syk is recruited to the phosphorylated ITAMs where it is also phosphorylated by Lyn (Turner et al., 2000). Syk then autophosphorylates followed by phosphorylation, by Syk, of tyrosine residues in many other target proteins in the cell.

B-CLL cells express low levels of surface BCR (Ternynk et al., 1974; Digherio et al., 1980; Matutes et al., 1994) and have been observed to have defective BCR signalling (Lankester et al., 1995), although there is heterogeneity in the extent of signalling observed. Signalling is higher in B-CLL cells where the I_gV_H genes are unmutated (Lanham et al., 2003; Chen et

al., 2002) as discussed further in section 1.3.4. Functional deficiency of the BCR may be due to a defect in CD79b, observed to have low expression in B-CLL cells (Zhomas et al., 1996; Thompson et al., 1997). Mutations in CD79b have been reported in B-CLL cells (Gordon et al., 2000), however another study could not detect the defect (Alfarano et al., 1999). Alternative splicing can produce a second CD79b transcript ΔCD79b, which results in the removal of exon 3 and the extracellular Ig-like domain (Hashimoto et al., 1995). This alternative transcript has been observed to be overexpressed in B-CLL cells (Alfarano et al., 1999; Payelle-Brogard et al., 2002; Cragg et al., 2002). ΔCD79b is expressed in normal activated B cells (Koyama et al., 1995) and can inhibit cell surface expression and signalling of BCR (Indraccolo et al., 2002; Cragg et al., 2002). An alternative explanation for low surface expression of BCR may be a post-transcriptional defect in assembly or trafficking of the BCR from the endoplasmic reticulum to the cell surface (Payelle-Brogard et al., 2002).

1.3 B-CLL subtypes

1.3.1 Prognostic factors

The clinical staging systems developed by Rai et al (1975) and Binet (1981) classify B-CLL into broad prognostic groups, as determined by clinical observations, and are used by clinicians for the determination of treatment course for a patient. However, these systems do not accurately predict the course of disease for each individual patient due to the considerable heterogeneity in progression of B-CLL observed. Therefore, other prognostic factors are required for determination of clinical course.

1.3.2 CD38 expression

CD38 expression has been observed to correlate with a more aggressive form of B-CLL (Damle et al., 1999), however subsequent studies have produced contrasting results. Some reports have agreed with the results of Damle et al and found CD38 expression to correlate with aggressive disease (Lin et al., 2002; Dürig et al., 2002; Chevallier et al., 2002; Morabito et al., 2002), however others have not observed any correlation (Thunberg et al., 2001; Domingo-Domenech et al., 2002). It has been suggested that CD38 expression may vary throughout the course of the disease (Hamblin et al., 1999; Chevallier et al., 2002), although there is also conflicting data for this observation (D'Arena et al., 2002).

Therefore, it has not been determined whether CD38 can be used as a reliable prognostic marker for B-CLL disease progression.

1.3.3 I_gV_H mutational status

B-CLL disease progression has also been observed to correlate with the mutational status of the immunoglobulin heavy chain-variable-region (I_gV_H) genes (Hamblin et al., 1999; Damle et al., 1999). B-CLL patients with unmutated I_gV_H genes have a more aggressive form of the disease and often have unfavourable cytogenetic features, such as 11q22-23 and 17q13 chromosome deletions, trisomy 12 and p53 dysfunction. In contrast patients with mutated I_gV_H genes have prolonged survival, and 13q14 chromosome deletions are frequently observed (Hamblin et al., 1999; Stilgenbauer et al., 2002; Lin et al., 2002). Therefore I_gV_H gene mutation status could provide a new prognostic marker. However, determination of the mutational status of I_gV_H genes would not be practical to perform on a routine basis so is not itself useful clinically.

Somatic hypermutation of I_gV_H genes occurs at the germinal centre stage of B cell development (Jacob et al., 1991; Klein et al., 1998). This suggested that those B-CLL cells with unmutated I_gV_H genes were derived from pre-germinal centre naive B cells whilst those with mutated I_gV_H genes were derived from memory B cells that had passed through the germinal centre and undergone somatic hypermutation, and therefore may represent two distinct diseases grouped together by common clinical characteristics. Two microarray studies were performed to address this question, which determined the gene expression profiles for B-CLL cells with mutated and unmutated I_gV_H genes (Klein et al., 2001; Rosenwald et al., 2001). Both studies revealed that the two subtypes share a common gene expression profile distinguishable from normal B cells and other types of B cell malignancy, therefore both do represent the same disease. These studies also revealed that both subtypes had a gene expression profile that appeared most related to memory B cells, although the difference in expression profiles for naïve and memory B cells is only small (Klein et al., 2003) so the hypothesised cell of origin for each subtype stated above is not ruled out by this observation. A number of differences in gene expression between the two subtypes were identified however. The most differentially expressed of these was zeta-chain-associated protein 70 (ZAP70). ZAP70 is a member of the syk-ZAP70 protein tyrosine kinase family and is normally expressed in T cells and natural killer cells, where it plays the same role in TCR signalling as syk does in BCR signalling (Chan et al., 1992;

Vivier et al., 1993), but is not usually expressed in B cells. Differential expression of ZAP70 between the B-CLL subtypes has also been observed at the protein level (Crespo et al., 2003). Analysis of ZAP70 levels would be relatively straightforward to perform on a routine basis, so may provide a valuable new prognostic marker for I_gV_H gene mutation status and therefore disease progression. However, it should be noted that ZAP70 levels do not correlate 100% with I_gV_H gene mutational status (Crespo et al., 2003; Orchard et al., 2004).

1.3.4 ZAP70 and BCR signalling

BCR signalling in B-CLL has been observed to be variable and shown to correlate with I_gV_H gene mutation status, where patients with unmutated I_gV_H genes have increased signalling (Chen et al., 2002; Lanham et al., 2003). Increased BCR signalling *in vivo* could lead to enhanced survival and/or proliferation of B-CLL cells leading to a worse prognosis (Kipps, 1997; Chen et al., 2004). As both ZAP70 and syk play the same role in BCR signalling, it was suggested that ZAP70 could enhance BCR signalling in B-CLL cells. Previous evidence suggested ZAP70 was an unlikely candidate for this role as B-CLL cells had been shown to express syk (Chen et al., 2002), and syk has 100-fold greater protein tyrosine kinase activity than ZAP70. Subsequent studies have found ZAP70 does directly enhance BCR signalling however (Chen et al., 2002; Chen et al., 2004). In fact, increased BCR signalling is observed in patients with high levels of ZAP70 but mutated I_gV_H genes (Chen et al., 2004), suggesting that ZAP70 could be an important prognostic marker in its own right, correlating with increased BCR signalling and poor prognosis.

1.4 Regulation of translation

1.4.1 Principles of translation control

Regulation of gene expression is a complex process and is exerted at multiple stages in the expression pathway, including translation. Translation, or protein synthesis, is energetically the most expensive process that occurs in the cell so must be tightly regulated (Rhoads, 1988; Rhoads et al., 1993) and there are now numerous examples of the use of translation control in a wide variety of biological processes. Translation can be regulated globally by altering the overall rate, which allows cells to respond rapidly to changes in intracellular or extracellular conditions without the requirement for new transcription. This type of control can be exerted by modulation of the translational machinery. However control can also be

exerted on specific messages or groups of messages without affecting the overall rate. The efficiency of translation for a specific mRNA depends on the properties of that message, and this can be increased or decreased by other factors.

1.4.2 Overview of translation

Translation is divided into three stages; initiation, elongation and termination. The first step, initiation is a complex process that results in the recruitment of the ribosome, as well as initiator-methionyl-transfer RNA (Met-tRNA$_i$) to the initiation codon of the mRNA. It is the rate limiting step in translation and therefore the stage at which the majority of regulation is exerted. A diagrammatical representation is shown in Figure 1.3.

Initiation can be subdivided into four separate steps. The first is the formation of a 43S pre-initiation complex, which consists of the small (40S) ribosomal subunit, the initiator tRNA (Met-tRNA$_i$) and a number of protein factors termed eukaryotic initiation factors (eIFs). Met-tRNA$_i$ binds to the 40S as part of ternary complex, which also contains eIF2 and GTP. Binding of the ternary complex is aided by eIF1, eIF1A and eIF3. With the addition of a further initiation factor eIF5, the 43S pre-initiation complex is formed.

The second step is the recruitment of the 43S pre-initiation complex to the 5'end of the mRNA, aided by another group of initiation factors that comprise the eIF4F complex. This contains three proteins; eIF4E, eIF4A and eIF4G. eIF4E directs recruitment by physically interacting with the m^{7}GpppN cap structure that is found on the 5' end of a mRNA, and with eIF4G (Marcotrigiano et al., 1997; Matsuo et al., 1997; Tomoo et al., 2002). eIF4A is a DEAD box helicase that can unwind RNA secondary structure and has a role in the next stage of translation initiation, scanning. eIF4G functions as a scaffold protein for the complex by interacting with the multiple factors involved (Hentze, 1997). eIF4E interacts with eIF4G so as to bridge the interaction with the 5'cap and its binding site is located in the N-terminal region of eIF4G. There are two binding sites for eIF4A, one in the central region and one in the C-terminal region (Imataka and Sonenberg, 1997). A binding site for PABP (poly(A) binding protein) has also been identified in this region of eIF4G (Le et al., 1997; Wells et al., 1998). PABP also associates with the poly(A) tail at the 3'end of the mRNA, therefore its interaction with eIF4G causes circularisation of the mRNA. This has been shown to enhance the affinity of eIF4F for the cap (Wei et al., 1998) and could potentially affect translation regulation by bringing regulatory elements in the 3'UTR into

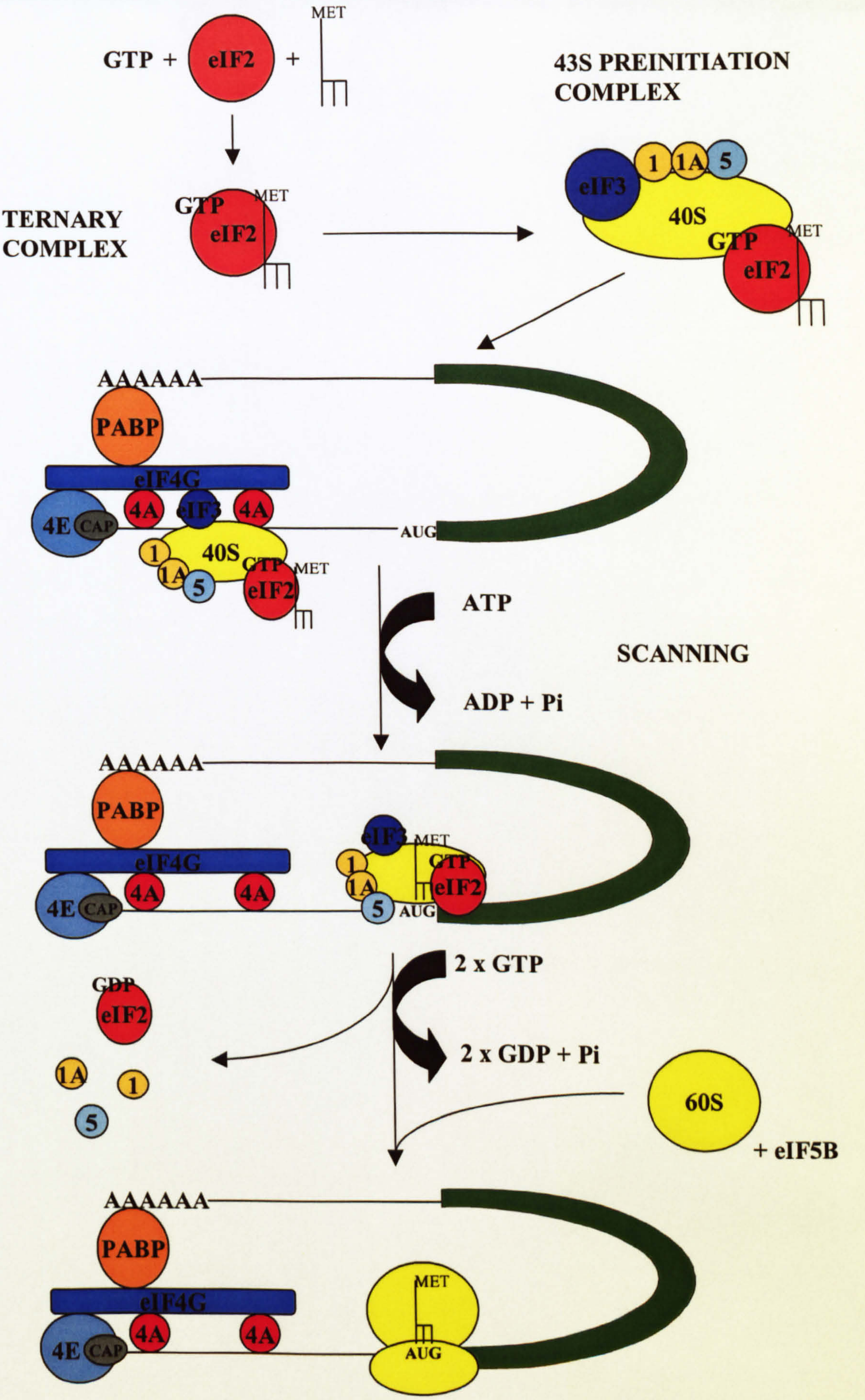


Figure 1.3 Cap-dependent translation initiation.

close proximity with the translation machinery. The 43S pre-initiation complex is recruited to the eIF4F complex through binding of eIF3 to the central domain of eIF4G, and is thus recruited to the mRNA 5'cap (Bommer et al., 1991).

Once the 40S subunit has been recruited to the mRNA, the translation initiation codon is located through a process termed scanning. The exact mechanism by which this occurs has not been deduced. Originally, a model was suggested in which the 40S subunit moved in a linear fashion along the mRNA until the initiation codon was reached (Kozak, 1999), however this has not been directly demonstrated. A recent report has provided some new insights into the process using purified ribosome subunits and initiation factors in a reconstituted system (Pestova and Kolupaeva, 2002). It was observed that a minimal complex containing the 40S subunit plus eIF3, eIF2 ternary complex and eIF1 could perform scanning and locate the initiation codon. In the absence of eIF1 the initiation codon could not be recognised. Initiation usually occurs at the most 5'AUG, although occasionally CUG or GUG are used (Kozak, 1989). However it has to be in good context, which depends on the surrounding sequence. The consensus sequence GCC(**A/G**)CC<u>AUG</u>**G** provides optimal conditions (Kozak, 1986; Kozak, 1987; Kozak, 1999). If eIF1 is absent from the scanning complex the 43S complex does not discriminate between an AUG in good context and one that isn't. The minimal scanning complex determined could only perform successful scanning on nonstructured 5'UTRs. When secondary structure was present, eIF4A, eIF4B and eIF4F were required. eIF4A is a DEAD box helicase which can unwind RNA secondary structure in the 5'UTR allowing the 43S complex to continue scanning (Lorsch and Herschlag, 1998). eIF4B and eIF4F improve the efficiency of the unwinding activity of eIF4A. On the basis of this evidence, Pestova and Kolupaeva suggested a model for the scanning process. They proposed that the 43S complex can have two confirmations: a 'closed' scanning incompetent form in the absence of eIF1 and an 'open' scanning competent form, which can recognise the translation initiation codon, in its presence, while eIF4A, eIF4B and eIF4F, plus eIF1 and eIF1A contribute to the processivity of the scanning process.

Once the initiation codon is located the 60S ribosome subunit joins the 40S subunit to form the 80S ribosome complex. When the 40S subunit reaches the initiation codon, base pairing between the codon and the Met-tRNA$_i$ anticodon occurs. Subsequently, the GTP bound to eIF2 undergoes hydrolysis to GDP, catalysed by eIF5. This is followed by the release of

eIF2-GDP and possibly other initiation factors, and is required for 60S subunit binding. A second GTPase activity of eIF5B is also required. GTP-bound eIF5B is required for 60S binding and once the 80S complex is formed it catalyses the hydrolysis of the GTP causing the release of eIF5B (Pestova et al., 2000; Lee et al., 2002). The 80S ribosome complex is then ready to begin elongation.

Elongation is the principal phase of translation, in which the polypeptide chain is synthesised. At the end of the initiation phase, the initiator tRNA is located in the P site of the ribosome and the A site is empty (Figure 1.4). In the first step of the elongation cycle, aminoacylated tRNA is brought to the A site as a ternary complex with elongation factor 1A (eEF1A) and GTP. Both correct (cognate) and incorrect (noncognate) aminoacyl tRNAs can enter the A site, however when the correct codon-anticodon interaction occurs conformational changes in the ribosome are induced, stabilising tRNA binding, and GTP is hydrolysed by eEF1A. This leads to the release of the aminoacylated end of A site tRNA by eEF1A. Peptide bond formation can then occur, catalysed by ribosomal peptidyl transferase, resulting in de-acetylation of the P site tRNA and transfer of the peptide chain to the A site tRNA. During the final step of the elongation, termed translocation, the de-acetylated tRNA is transferred to the E site and peptidyl tRNA to the P site, aided by elongation factor 2 (eEF2). This leaves the A site empty and ready to receive the next cognate ternary complex for the start of another elongation cycle (Figure 1.4). (Reviewed in Kapp and Lorsch, 2004).

The elongation cycle continues until a termination codon is reached, which is either a UAA, UAG or UGA codon, and is recognised by release factor 1 (eRF1) instead of an aminoacyl tRNA. The presence of eRF1 in the ribosome A site leads to hydrolysis of the ester bond between the polypeptide chain and the P site tRNA, allowing the polypeptide to be released, a reaction believed to be catalysed by peptidyl transferase (Figure 1.5). Another release factor, eRF3 is also involved in termination; it is a GTPase and stimulates the activity of eRF1. (Reviewed in Kapp and Lorsch, 2004).

1.4.3 Regulation of translation by regulatory factor modifications

Modifications to the regulatory factors involved in translation are important mechanisms utilised to control translation. These modifications usually result in global changes in translation, although can also regulate specific mRNAs.

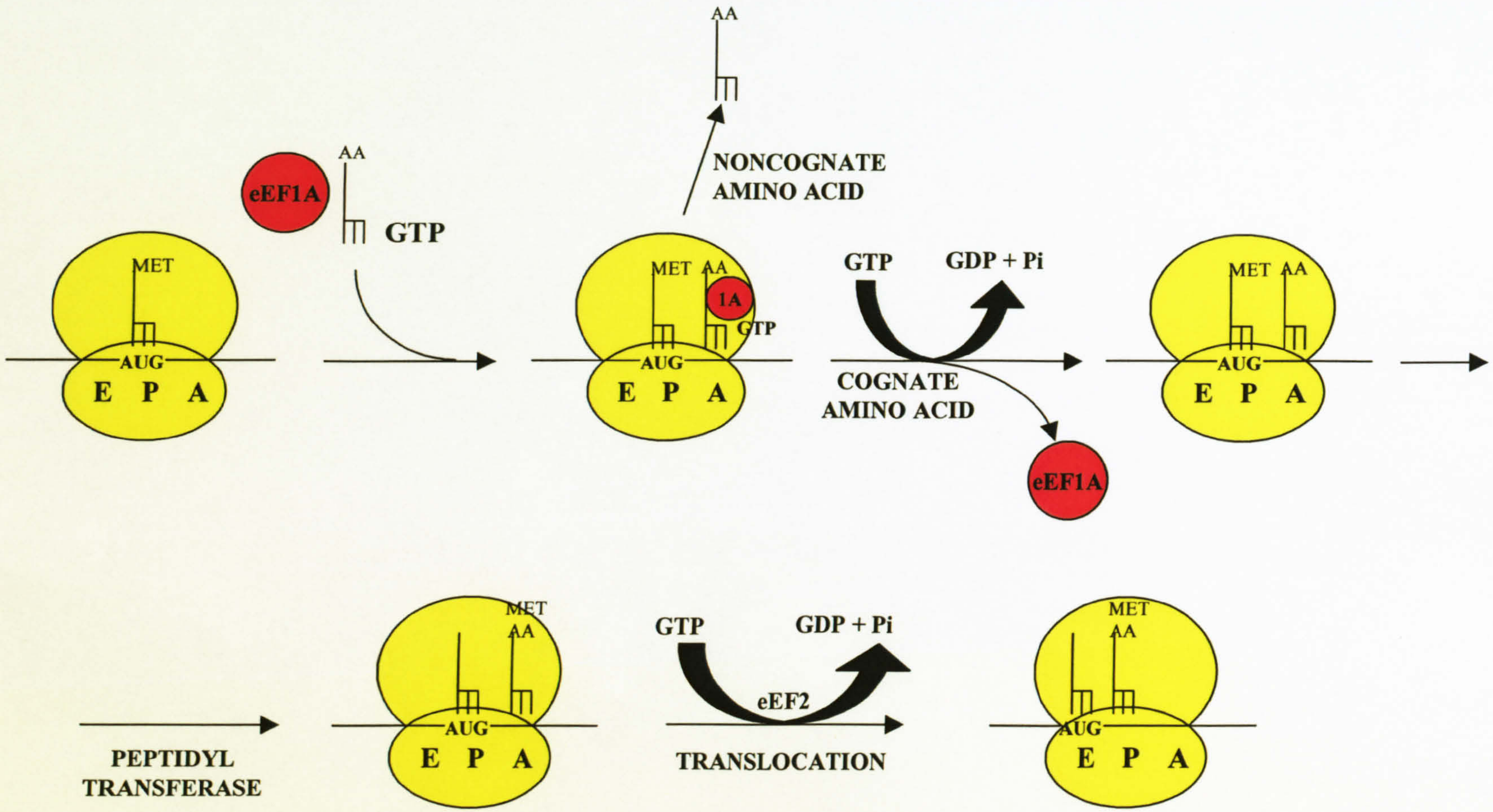


Figure 1.4 Translation elongation cycle

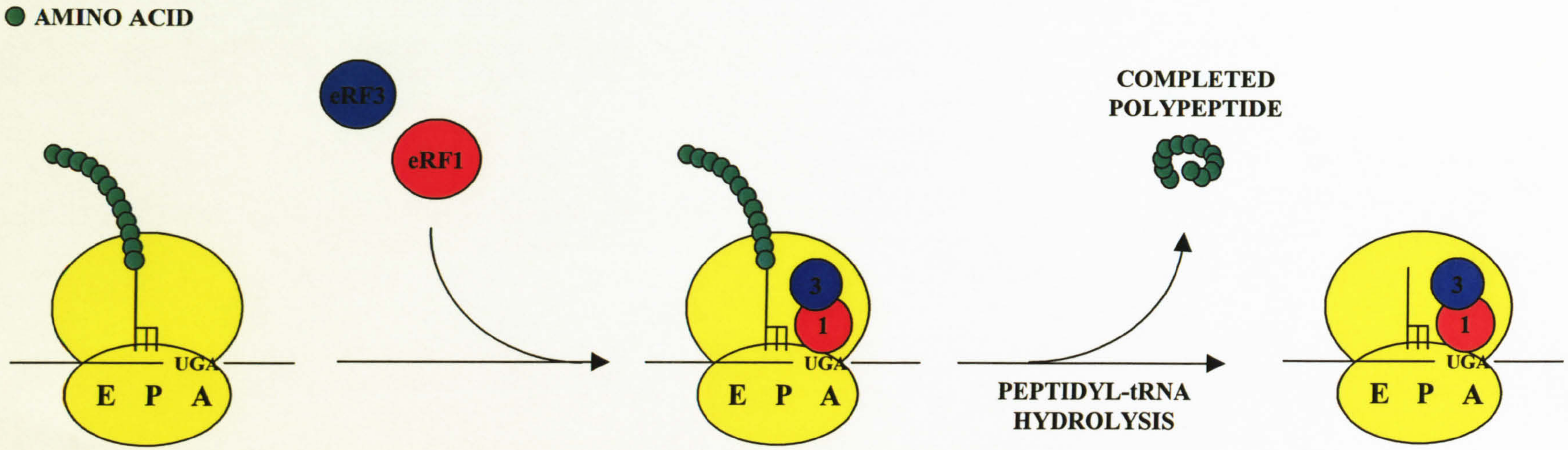


Figure 1.5 Translation Termination

Regulation of eIF2 by phosphorylation is an important point of control in translation initiation. During the initiation process, the GTP molecule bound to eIF2 in the ternary complex is hydrolysed to GDP. This GDP bound form of eIF2 cannot bind Met-tRNA$_i$, therefore, for a subsequent round of initiation, the GDP must be exchanged for GTP again. This is catalysed by the guanine nucleotide exchange factor (GEF) eIF2B (Hinnebusch, 2000). eIF2 has three subunits; α, ß and λ. eIF2α is the regulatory subunit and is subject to phosphorylation on serine 51, which blocks the GTP-exchange reaction by reducing the dissociation rate of eIF2 from eIF2B (Kimball et al., 1998) (Figure 1.6). eIF2B levels in the cell are lower than those of eIF2 so this effectively sequesters all eIF2B (Rowlands et al., 1988; Oldfield et al., 1994), preventing further GDP-GTP exchange from occurring and thus inhibiting translation initiation. There are a number of kinases, activated under different conditions, which can phosphorylate eIF2α. These include the haem-regulated inhibitor (HRI), stimulated by haem depletion, GCN2 (general control non-derepressible-2) activated by amino acid starvation, PKR (protein kinase activated by double-stranded RNA) activated by viral infection and PERK, which is stimulated by conditions inducing endoplasmic reticulum (ER) stress (reviewed in Chen, 2000; Kaufman, 2000; Ron and Harding, 2000). Phosphorylation of eIF2α results in reduction of global translation rates, however it can also result in activation of specific mRNAs as will be discussed below in section 1.4.5.

Another important step for control of translation initiation is the regulation of eIF4E. eIF4E is required for recruitment of the 43S pre-initiation complex to the mRNA 5'cap as it binds to the cap and eIF4G. The eIF4E binding domain in eIF4G is also found in a family of proteins known as the 4E-binding proteins (4E-BPs). They can bind to eIF4E, preventing binding to eIF4G and its participation in translation initiation (Haghighat et al., 1995; Rau et al., 1996). 4E-BPs bind to eIF4E under normal cell conditions when they are in a hypophosphorylated form. Phosphorylation causes release of 4E-BPs from eIF4E so that it can bind to eIF4G (Pause et al., 1994), usually performed by mTOR (Figure 1.7). Phosphorylation is induced by a number of extracellular stimuli, including growth factors, hormones, amino acids and cytokines. This leads to the activation of the PI-3K signalling pathway. For phosphorylation of 4E-BPs, PI-3K signals through protein kinase B (PKB/Akt), which activates mTOR (reviewed in Gingras, 1999). Recent evidence has suggested that other enzymes, such as Pim-2, may also be involved in 4E-BP phosphorylation (Fox et al., 2003). Phosphorylation of eIF4E itself may also contribute to

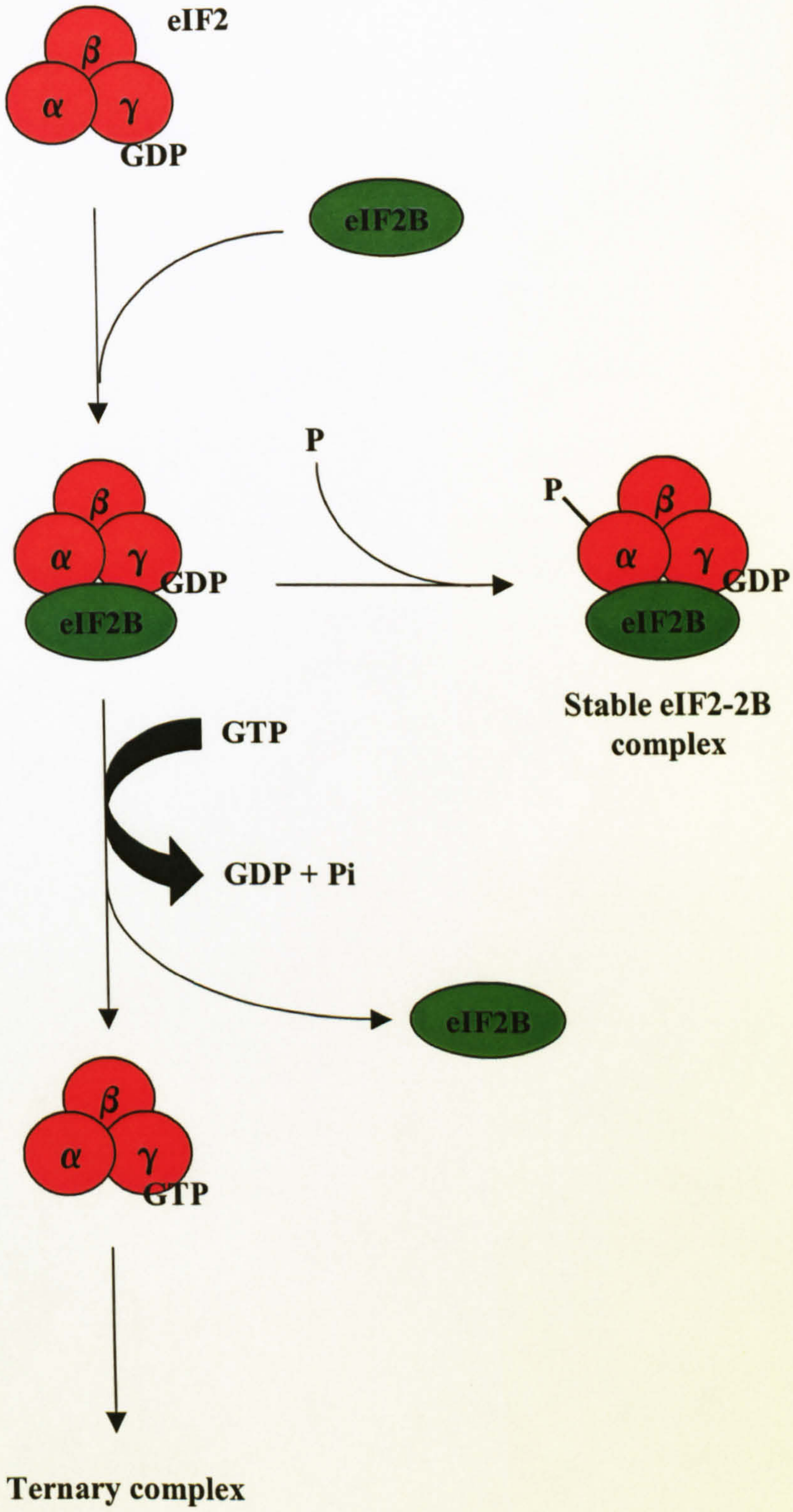


Figure 1.6 Regulation of translation by phosphorylation of eIF2α. The phosphorylation of the α subunit of eIF2 prevents guanine nucleotide exchange by stabilising the eIF2-GDP-eIF2B complex. Formation of ternary complex is therefore prevented leading to an inhibition of translation.

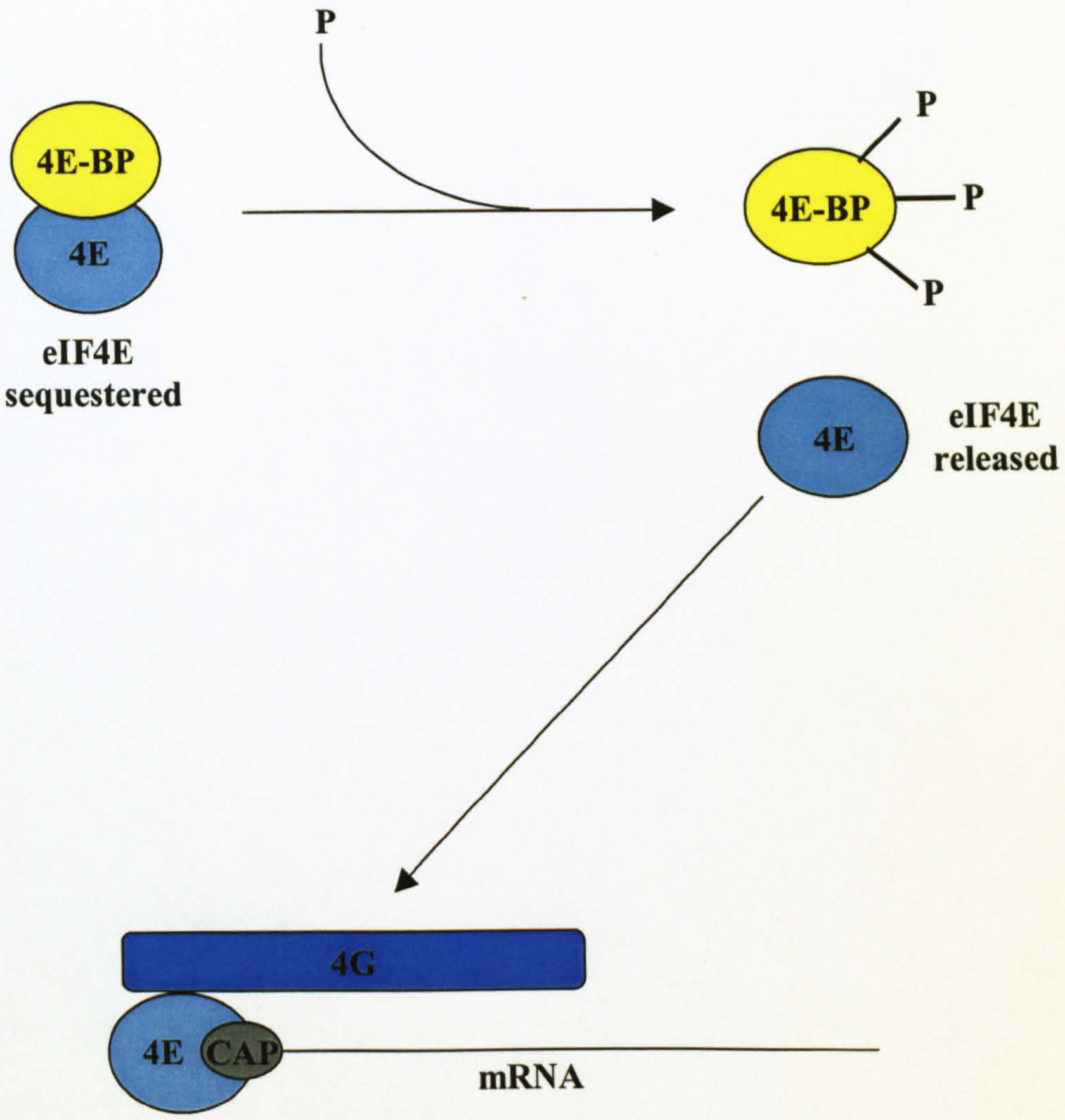


Figure 1.7 Regulation of translation by 4E-BPs. The 4E-BPs bind to eIF4E preventing its interaction with eIF4G and the mRNA cap thus inhibiting translation initiation. Phosphorylation of the 4E-BPs leads to release of eIF4E, allowing its participation in translation.

regulation. eIF4E is phosphorylated by the MAP kinase-interacting protein kinase (Mnk) 1 and 2 in response to stimuli such as growth factors and mitogens, via signalling through ras (Waskiewiczs et al., 1999; Gingras, 1999). It doesn't directly interact with eIF4E but binds to eIF4G bringing the kinase and substrate into close proximity during translation initiation (Pyronnet et al., 1999). The resulting phosphorylation of eIF4E has been observed to correlate with increased translation rates (Scheper and Proud, 2002) and is thought to act by increasing cap-binding affinity (Minich et al., 1994). However, recent reports have provided contradictory evidence that questions this function (Zuberek et al., 2003; Scheper et al., 2002).

The elongation phase is also subject to regulation by modulation of translation factor activity. eEF2, required for the translocation step (Figure 1.4), can be regulated by phophorylation. Its activity is inhibited by phosphorylation catalysed by eEF2 kinase, which prevents it binding to the ribosome (Carlberg et al., 1990; Nairn and Palfrey., 1987) and consequently inhibits translation elongation. Dephosphorylation is catalysed by the phosphatase PP2A (Redpath and Proud, 1990).

1.4.4 5'TOP mRNAs

The expression of many mammalian proteins associated with the translational apparatus is regulated at the level of translation in response to growth conditions. This group of messages includes ribosomal proteins, eEF1A, eEF2 and PABP, which are characterised by the presence of a 5' oligopyrimidine tract in the 5'UTR of their mRNAs and are referred to as 5'TOP mRNAs. This is a *cis*-regulatory element consisting of a C residue at the cap followed by a stretch of 4-14 pyrimidine residues. Under conditions of growth 5'TOP messages are actively translated but in quiescent cells their translation is shut off, conditions where newly synthesised translational machinery would not be required (Jefferies et al., 1994). Phosphorylation of ribosomal protein S6 (RPS6), in response to PI-3K signalling, has been implicated in the regulation of 5'TOP translation. Mitogenic stimulation causes activation of the PI-3K signalling pathway, which leads to the phosphorylation of RPS6 by S6 kinases. This has led to the suggestion that this increased the affinity of ribosomes for 5'TOP mRNAs. Treatment of cells with rapamycin, which blocks RPS6 phosphorylation by S6 kinases, was also shown to repress translation of 5'TOP mRNAs (Terada et al., 1994; Shima et al., 1998; Kawasome et al., 1998). In addition transfection of cells with mutant S6 kinases also repressed translation of 5'TOPs

following mitogenic stimulation (Jefferies et al., 1997). However, recent studies have put this observation into doubt. Activation of 5'TOP mRNA translation in response to growth signals was shown to be dependent upon PI-3K signalling, but phosphorylation of RPS6 by S6 kinases was not required (Stolovich et al., 2002).

1.4.5 Upstream AUGs and open reading frames (uORFs)

For a number of mRNAs (about 10%), the first AUG encountered during scanning is not the initiation codon for the coding ORF. These upstream AUGs (uAUG) reduce the efficiency of translation and are used as a mechanism for control (Kozak, 1991). Sometimes the uAUG is succeeded by an in frame-termination codon so has potential to produce a short peptide and is termed an upstream open reading frame (uORF). There are two mechanisms that have been described that can explain the translation of the coding ORF downstream of uAUGs, or uORFs. The upstream AUGs are sometimes not recognised by all scanning ribosomes, which therefore bypass the uAUGs and continue scanning until they reach the initiation codon of the main ORF. This occurs when the AUG codons are not in good context and is termed 'leaky scanning'. A rarer mechanism is re-initiation, where the 40S ribosomal subunit remains attached to the mRNA after translation of an uORF and continues to scan to the coding ORF. In general, this is not an efficient mechanism and is only possible if the uORFs are very short in length. Increasing the distance between uORF and coding ORF can also increase the efficiency of the mechanism by allowing more time for reloading of ternary complex. (Reviewed in Morris and Gebaulle, 2000; Meijer and Thomas, 2002).

A well characterised example of the use to re-initiation is the yeast GCN4 mRNA. This encodes a protein that functions as a translational activator of genes that regulate amino acid biosynthesis. Translation of GCN4 is regulated by amino acid availability using the mechanism of re-initiation. The 5'UTR of GCN4 mRNA contains four uORFs, the first and fourth being important for regulation. uORF1 allows re-initiation of translation whereas uORF4 promotes dissociation of 40S ribosome subunits due to the GC rich sequence that surrounds the termination codon. When amino acids are plentiful, the 40S subunit is readily recharged with active ternary complex after translation of uORF1, as it scans between uORF1 and uORF4. Therefore, the majority of 40S subunits will initiate translation at uORF4 and will not reach the GCN4 ORF. Under conditions of amino acid deprivation eIF2α is phosphorylated by GCN2, reducing the amount of active ternary complex

available. Therefore 40S subunits are less likely to have been recharged with active ternary complex when they reach uORF4 so it is bypassed. However, due to the longer scanning time, recharging may have occurred by the time the GCN4 ORF is reached allowing efficient translation and thereby providing a mechanism by which GCN4 translation can be up-regulated when an increase in amino acid biosynthesis is required (Hinnebusch, 1997).

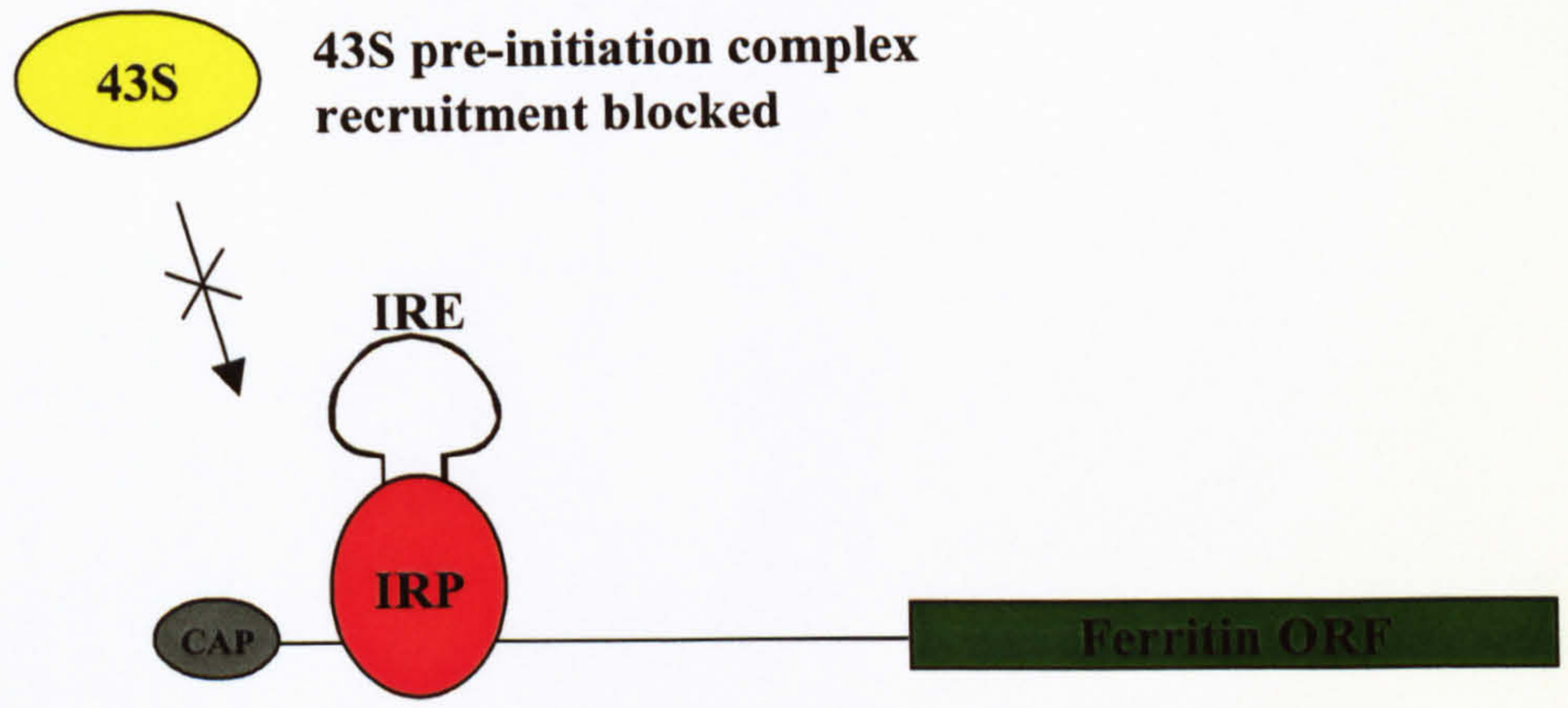
1.4.6 *Trans*-acting factors

Translation of specific mRNAs can be regulated by the binding of proteins to specific sites in the message. A good example is the regulation of ferritin heavy- and light-chain translation by iron regulatory proteins (IRP) 1 and 2. Ferritin is involved in iron storage, thus translation is repressed under conditions when iron levels are low. The mRNAs for these two proteins contain a binding site for IRP1 and IRP2 in the 5'UTR called the iron response element (IRE). It is found within 40 nucleotides of the mRNA 5'cap. When IRP1 and 2 bind to the IRE, recruitment of the 43S pre-initiation complex is blocked (Figure 1.8) (Gray and Hentze, 1994; Muckenthaler et al., 1998). The position of the IRE with respect to the 5'cap is important as an alteration in position, further from the cap abolishes IRP mediated regulation (Goosen et al., 1990; Paraskeva et al., 1999).

1.4.7 Secondary structure

The majority of mRNAs have 5'UTRs that are less than 200 nucleotides long. However a subset are characterised by long 5'UTRs that are highly structured and contain multiple AUG triplets (Kozak, 1991), which is inhibitory to scanning. The inhibitory effect of secondary structure is dependent on its position relative to the 5'cap. When a stem loop structure, of –30 kcal/mol free energy, was placed 12 nucleotides downstream of the cap translation initiation was inhibited, but when placed 52 nucleotides downstream translation could occur (Gray and Hentze, 1994). Positioning of the stem loop in close proximity to the cap prevented 43S pre-initiation complex binding whereas inhibition was abolished when the stem loop was moved further downstream. If a more stable stem loop, of –61 kcal/mol, was inserted 71 nucleotides downstream, translation initiation was inhibited as the scanning 43S complex was unable to unwind the structure. Certain mRNAs with highly structured 5'UTRs, inhibitory to scanning, can be translated efficiently under conditions when other such messages are not. There are two mechanisms that have been discovered by which translation of such mRNAs can be mediated; ribosomal shunting and internal ribosome entry.

A: - Fe conditions



B: + Fe conditions

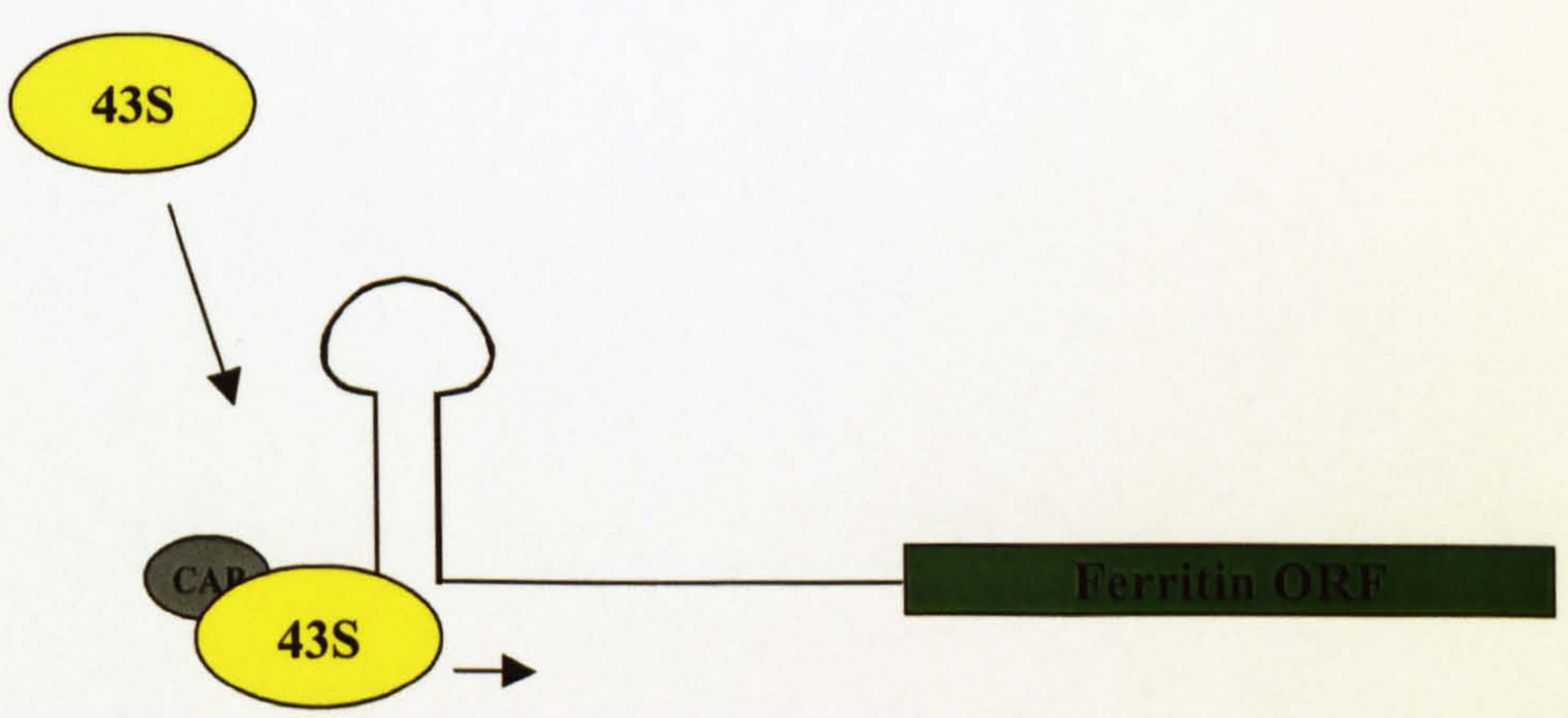


Figure 1.8 Regulation of ferritin translation by iron. When iron levels are low, iron binding protein (IRP) binds to the iron response element (IRE) in the 5'UTR of ferritin mRNA, blocking 43S pre-initiation complex binding to the 5'cap thus inhibiting translation (A). When iron levels are high this inhibition is released (B).

Ribosomal shunting is an unusual mechanism that was initially observed to mediate the translation initiation for a number of viral mRNAs, including cauliflower mosaic virus 35S mRNA (Futterer et al., 1993) and adenovirus late mRNAs (Yueh and Schneider, 1996), plus may be a mechanism used by some mammalian mRNAs (Yueh and Schneider, 2000; Rogers et al, 2004). Translation initiation begins in the same way as the canonical scanning method with recruitment of the 43S pre-initiation complex to the 5'cap and the induction of scanning. However, when the scanning complex reaches a stable RNA structure, it is stalled and dissociates from the mRNA, followed by intramolecular shunting to a downstream landing site the other side of the structure, where it resumes scanning until the initiation codon is reached (Figure 1.9). The exact mechanism of ribosomal shunting has not been deduced, however, for the adenovirus late mRNAs, sequence complementarity to the 18S rRNA may be involved. The 5'UTRs of adenovirus late mRNAs contains a series of hairpin structures that form large single stranded loops (Zhang et al., 1989; Dolph et al., 1990). Several of the hairpins possess sequence complimentarity to the 18S rRNA, which may function by binding the 18S rRNA to aid shunting (Yueh and Schneider, 2000). The 5'UTRs of the human heat shock protein 70 (hsp70) and c-*fos* also show complementarity to 18S rRNA and can be translated by shunting (Yueh and Scnieder, 2000). Under conditions of heat shock, scanning of these mRNAs was completely inhibited, but shunting was unaffected thereby providing a mechanism allowing translation of these messages under conditions where normal cap-dependent scanning was reduced.

Internal ribosome entry allows translation initiation to occur in a cap-independent manner by direct recruitment of the ribosome to a complex secondary structural element in the 5'UTR, termed an internal ribosome entry site (IRES) (Figure 1.10). This mechanism allows the ribosome to bypass secondary structure that may be inhibitory to scanning and any uAUGs that are present. IRESs were first discovered in a family of viruses, the picornaviruses (Jang et al., 1988; Pelletier and Sonenberg, 1988). Internal ribosome entry is used for translation of all their genes and their mRNAs have no 5'cap. Upon cellular infection, eIF4G is cleaved by a viral protease, separating the 4E binding section from the rest of the protein and thereby inhibiting cap-dependent translation. However, translation of the viral mRNAs can still occur by IRES mediated translation (reviewed (Jackson and Kaminski, 1995; Hellen and Sarnow, 2001). IRESs have subsequently been discovered for numerous cellular genes that have long highly structured 5'UTRs. It is currently estimated

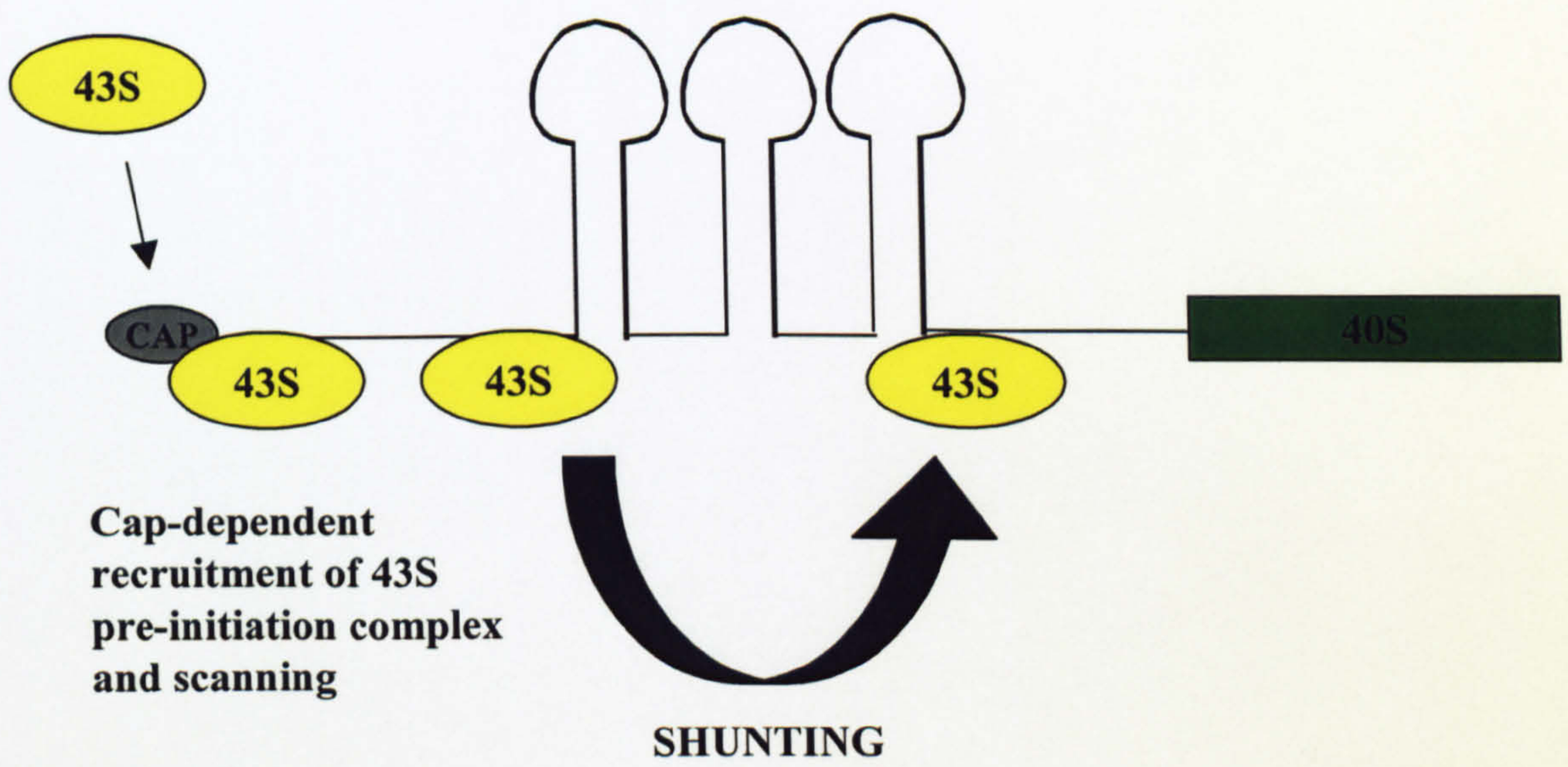


Figure 1.9 Ribosome shunting. Secondary structure in the 5'UTR is by passed by ribosome shunting, where the scanning 43S pre-initiation complex dissociates from the mRNA then binds again downstream of the secondary structure.

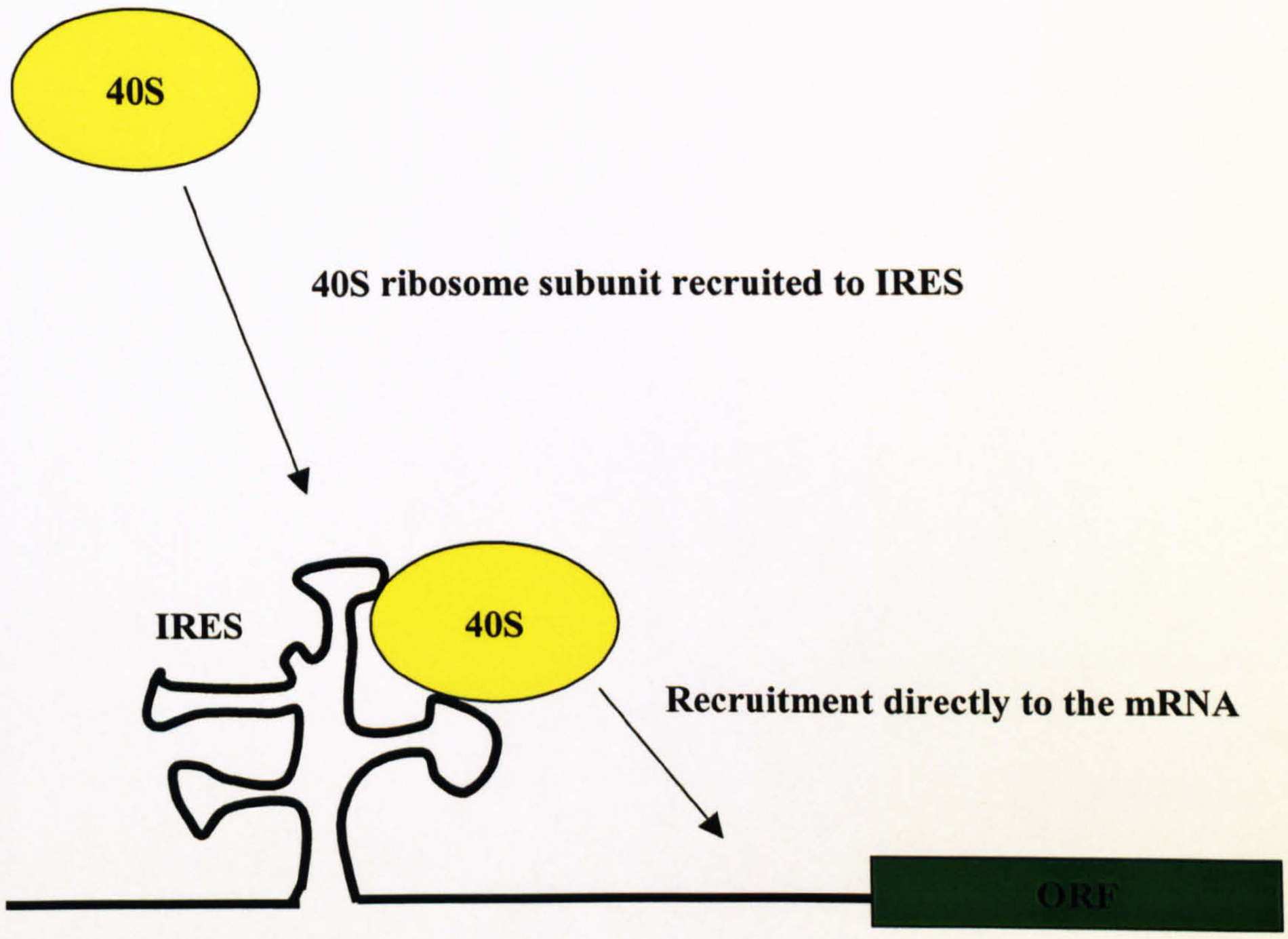


Figure 1.10 Internal ribosome entry. Translation initiation occurs in a cap-independent manner via recruitment of the ribosome to an IRES element.

that around 10% of mRNAs in the genome contain IRESs elements (Johannes et al., 1999; Qin and Sarnow, 2004).

The structures for viral IRESs have been relatively well defined and have been observed to be phylogenetically conserved. Recruitment of the ribosome is thought to occur via multiple interactions of the RNA secondary structure of the IRES with components of the translational apparatus. The ribosome is then either directly recruited to the initiation codon, or subsequent scanning may be required 3' of the IRES element to reach the translation start site. Data for cellular IRESs is not so extensive. The structure of cellular IRESs are less well defined and to date, no conservation of structure has been determined (LeQuesne et al., 2001; Bonnal et al., 2003; Jopling et al., 2004; Mitchell et al., 2003; Yaman et al., 2003), and the exact mechanism of ribosome recruitment has not been deduced (reviewed (Stonely and Willis, 2004).

There is growing evidence for the use of internal ribosome entry for translation of genes that are required to be expressed under conditions of cell stress when cap-dependent translation is reduced. For example, during apoptosis cap-dependent translation is inhibited due to the cleavage and/or modification of several translation initiation factors (Clemens et al., 2000). However, the translation of a number of proteins has been observed to be maintained via the use of internal ribosome entry. These are c-myc, DAP5, XIAP and PKCδ (Stoneley et al., 2000; Henis-Korenblit et al., 2000; Holcik et al., 2000; Morrish and Rumsby, 2002). A recent study has discovered that an IRES is present in the 5'UTR of the mRNA encoding Bcl2 (Sherrill et al., 2004), which was found to be active under conditions of cell stress induced by sodium arsonate or etoposide.

1.4.8 MicroRNAs

Regulation of translation by small RNA molecules, known as microRNAs (miRNAs), has recently been recognised as another important mechanism for control. The first microRNA discovered was *lin-4* (Lee et al., 1993; Wightman et al., 1993), which has subsequently been observed to have a crucial role in the regulation of developmental timing in *C. elegans* (Carrington and Ambros, 2003). Since this initial discovery, several hundred more miRNAs have been described in plants and animals, which are involved in a wide variety of biological processes including cell growth and apoptosis, highlighting this as an important mechanism of regulation.

Production of miRNAs occurs through cleavage of their primary transcripts by two RNases of the RNase III superfamily. The first cleavage takes place in the nucleus and is performed by the RNase Drosha, which produces precursors of ~70nts (Lee et al, 2003). The second cleavage takes place in the cytoplasm and is performed by the RNase Dicer (Hutvagner et al., 2001; Ketting et al., 2001; Grishok et al., 2001). The final miRNA is about 22 nucleotides in length and hybridises by incomplete base-pairing, usually at several sites within the 3'UTR of target mRNAs, causing a repression of translation. MiRNAs are functionally indistinguishable from small interfering RNAs (siRNAs). However, these RNA molecules bind with perfect complementarity to their targets mRNAs, which leads to degradation of the message (Zamore, 2002).

The mechanism by which miRNAs repress translation has not yet been determined. Repression of *lin-14* mRNA translation by the miRNA *lin-4* in *C. elegans* did not alter the association of the message with polysomes, indicating that *lin-4* repressed translation at the elongation or termination of translation (Olsen and Ambros, 1999) (Figure 1.11). However, it is not known by what mechanism this could occur, or if it is widely used by other miRNAs.

1.5 Deregulation of translation in tumorigenesis

1.5.1 Translation control and tumorigenesis

The various mechanisms discussed above, utilised to control translation can become deregulated in tumorigenesis. This may result in an increase in global translation rates, which is associated with rapid proliferation. Alternatively specific messages that encode oncogenes or tumour suppressors may become de-regulated.

1.5.2 Translation factors

Modification of the translation machinery may contribute towards tumorigenesis and can lead to an increase in the rate of protein synthesis. There are numerous examples in tumours of deregulated protein synthesis and expression of translation factors. Aberrant expression of ribosomal proteins has been observed in a number of cancers, which can lead to defects in the protein machinery. For example, RPL35A has been observed to be overexpressed in brain tumours and hepatocarcinomas (Lopez et al., 2002; Chung et al., 2002) and RPS16 in prostate cancer (Karan et al., 2002).

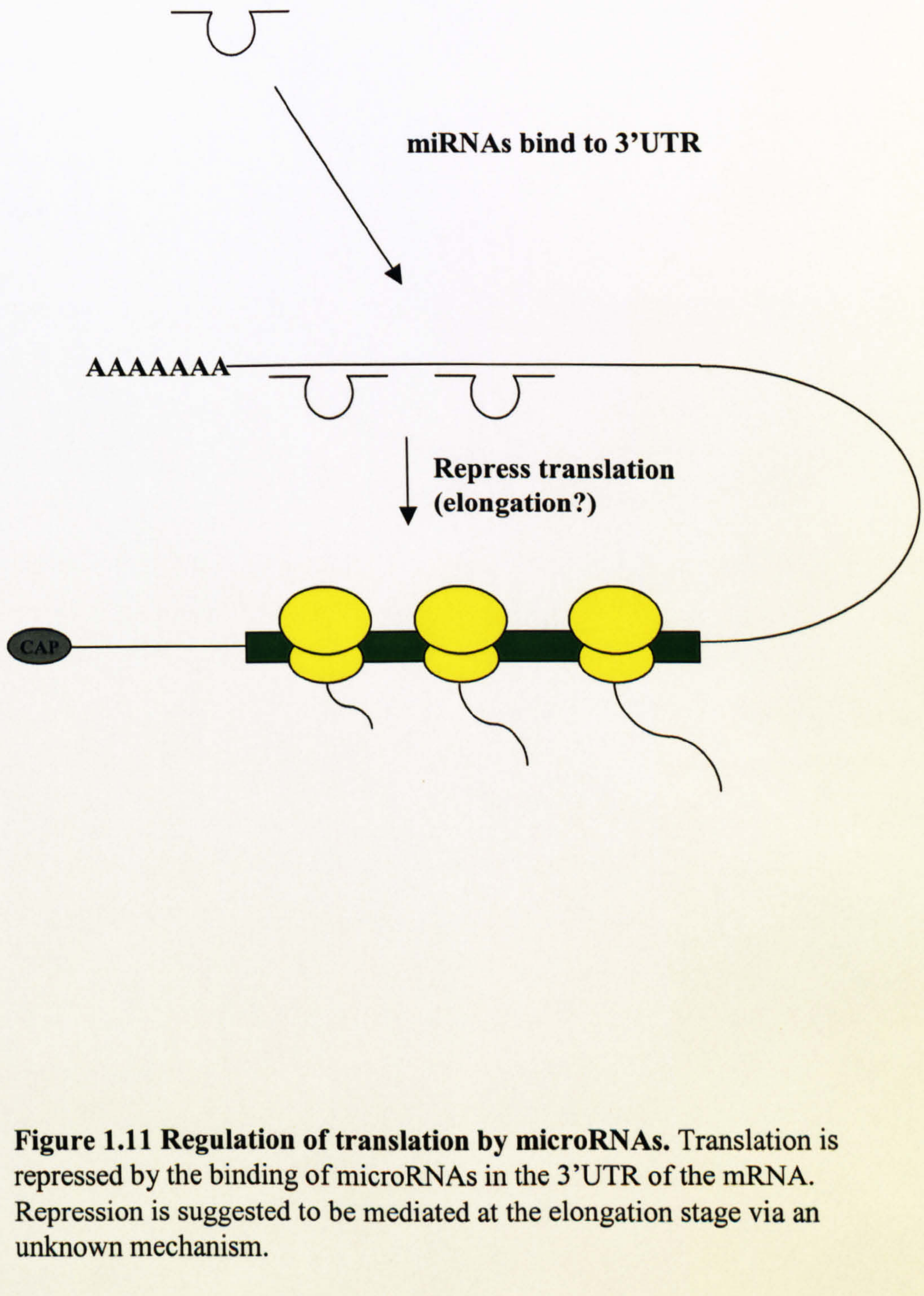


Figure 1.11 Regulation of translation by microRNAs. Translation is repressed by the binding of microRNAs in the 3'UTR of the mRNA. Repression is suggested to be mediated at the elongation stage via an unknown mechanism.

Abnormal regulation of translation regulatory factors has been observed in multiple types of cancer. There are two stages of the initiation process that are particularly implicated in tumorigenesis; ternary complex formation and recruitment of 43S pre-initiation complex to the 5'cap. eIF2 protein levels have been observed to be higher in tumour cells, for example eIF2α was observed to be elevated in Non-Hodgkins Lymphomas compared to in normal B cells and correlates with disease aggression (Wang et al., 1999). De-regulation of eIF2α phosphorylation may also contribute to tumorigenesis. Expression of a mutant unphosphorylatable form of eIF2α caused malignant transformation of NIH 3T3 cells (Donze et al, 1995). A reduction in the kinases that phosphorylate eIF2α could also have the same effect. A reduction in HRI (haem regulated inhibitor) has been observed in epithelial ovarian cancers (Hwang et al., 2000).

Recruitment of the 43S pre-initiation complex to the mRNA cap is also a critical step in the initiation process and as such, initiation factors involved have been found to be de-regulated in cancer. The cap binding protein, eIF4E has been observed to be overexpressed in numerous cancer types, including breast and prostate cancer, and Hodgkin's Lymphoma. Overexpression of eIF4E in NIH 3T3, CREF and MM3MG cell lines induced transformation and tumorigenesis (De Benedetti and Rhoads, 1990; De Benedetti et al., 1994; Lazaris-Karatzas et al., 1990; Li et al., 2001). An increase in translation was induced, however it was found that translation of only a select group of mRNAs was greatly enhanced, whilst only a slight increase was observed for the rest. This group of messages included genes that, under normal cellular conditions, are inefficiently translated due to the presence of long GC rich and highly structured 5'UTRs. The abundance of eIF4F complex is usually limiting due to the limited expression of eIF4E and binding of eIF4E to 4EBPs (Sonenberg et al., 1994; Gingras et al., 1999). However, when eIF4E levels are elevated, extra eIF4F complex is available resulting in increased translation of these mRNAs. Translation of other mRNAs is only slightly increased as their translation is relatively efficient under normal conditions. The mRNAs with long GC rich, structured 5'UTRs are often oncoproteins and growth or survival factors, therefore an increase in their expression would contribute to tumorigenesis. For example, the protooncogene c-*myc* has a long GC rich 5'UTR, which is inhibitory to translation initiation (Saito et al., 1983; Darveau et al., 1985). However, overexpression of eIF4E in CHO or CREF cells resulted in an increase in c-*myc* translation (De Benedetti et al., 1994). An increase in the availability of eIF4E could also result from a decrease in the level of 4EBPs or an increase in the phosphorylation of

these factors and hyperphosphorylation of 4E-BPs has been observed in tumours (Wu et al., 1998).

1.5.3 Deregulation of specific messages

Expression of specific proteins may become deregulated in tumorigenesis at the level of translation and this has been observed for a number of proteins via some of the mechanisms discussed above. Mdm2 is an oncoprotein, which can inhibit the actions of p53 and therefore block cell cycle arrest and apoptosis (Momand et al., 1992). The 5'UTR of mdm2 mRNA contains two uORFs, which inhibit translation (Brown et al., 1999). However, an alternative transcript is also produced that does not contain these uORFs, S-mdm2, and is therefore translated efficiently (Brown et al., 1999). S-mdm2 is usually only expressed at low levels in normal cells, however it has been observed to be elevated in tumour cells (Brown et al., 1999; Landers et al., 1997; Landers et al., 1994; Capoulade et al., 1998). Expression of the cyclin dependent kinase cdk4 can also be translationally regulated, via the actions of p53. It has been observed that p53 binds to the 5'UTR of cdk4 mRNA and inhibits its translation, in response to TGFß (Ewen et al., 1995; Miller et al., 2000). Therefore loss of p53 in tumours would result in loss of translational repression of cdk4, which may contribute to tumour progression.

Of the mRNAs where IRES elements have been identified, many of them encode proteins that are involved in cell growth, proliferation, apoptosis and angiogenesis. As many of these proteins are deregulated in tumorigenesis it is possible that aberrant IRES mediated translation may contribute. For example, c-Myc translation via internal ribosome entry is enhanced in multiple myeloma. c-Myc expression is regulated at multiple levels including at the level of translation. This protein can be translated by cap-dependent translation or in a cap-independent manner via an internal ribosome entry site (De Benedetti et al., 1994; Stoneley et al., 1998). c-Myc protein levels were observed to be elevated 20-fold in multiple myeloma and this was shown to be due to enhanced translation (Paulin et al., 1996). A single mutation of C to U in the 5'UTR of c-*myc* was found to correlate with this enhanced translation (Paulin et al., 1996) and was found to enhance IRES mediated translation in multiple myeloma (Chappell et al., 2000).

1.6 Aims of project

The evidence above reveals the importance of studying translational regulation in cancer. Gene expression in B-CLL cells has not previously been analysed at this stage therefore the overall aim of this project was to determine the translation status of these cells.

cDNA microarray analysis of gene expression is a useful tool used to screen the expression of multiple genes simultaneously and it is possible to study gene expression at the level of translation using this tecnique by separating mRNAs into translated and untranslated pools, as determined by polysome association (Johannes et al., 1999). cDNA microarray analysis was performed for B-CLL cells to create a gene expression profile at the level of translation, and has identified differences in gene expression relevant to the biology of the disease. Further analysis was then performed to analyse the mechanism of translation de-regulation and functional consequences of changes observed, with the possible identification of new treatment targets.

Chapter 2

Materials and Methods

2.1 General Reagents

Unless otherwise stated all chemical reagents were of analytical grade and were obtained from BDH laboratory supplies (Lutterworth, Leicestershire), Fisher Scientific (Loughborough, Leicestershire), ICN Flow Ltd (Thame, Oxfordshire), Sigma Chemical Company Ltd (Poole, Dorset), Oxoid (Unipath, Basingstoke, Hampshire), Pierce (c/o Perbio Science UK, Tattenhall, Cheshire) or BIO101 (c/o Anachem, Luton, Bedfordshire). Products for molecular biology were routinely purchased from Calbiochem (c/o CN Biosciences UK, Beeston, Nottingham), Gibco-BRL (Paisley, Scotland), Invitrogen (Paisley, UK), MBI Fermentas (c/o Helena Biosciences, Sunderland, Tyne and Wear), New England Biolabs (NEB) (c/o CP labs, Bishops Stortford, Hertfordshire), Pharmacia Biotech (Milton Keynes, Buckinghamshire), Promega (Southampton), QIAGEN (Crawley, West Sussex), Roche UK Ltd (Lewes, East Sussex) and Stratagene Ltd (Cambridge). Radiolabelled chemicals were purchased from Amersham International (Little Chalfont, Buckinghamshire) and NEN Dupont (Hounslow, London).

2.2 Tissue Culture Techniques

2.2.1 Media and supplements

DMEM medium: Dulbecco's modified eagle medium, without sodium pyruvate (Sigma)

RPMI 1640 medium: Rose Park Memorial Institute medium, with L-Glutamine (Sigma)

Medium was supplemented with foetal calf serum (FCS) (Helena Biosciences), L-glutamine (Sigma) and penicillin/streptomycin solution (Sigma) as indicated in table 2.1.

2.2.2 Maintenance of cell lines

The cell lines in table 2.1 were cultured in the growth medium indicated in sterile plasticware (TPP c/o Helena Biosciences) and grown at 37°C in a humidified atmosphere containing 5% CO_2. Adherent cells were grown to confluency in 75cm^2 flasks then detached using 1x Trypsin/0.5mM EDTA (Gibco BRL). Approximately 6.5x10^5 cells were diluted into fresh medium and re-plated into a new flask. Cells grown in suspension were maintained at a density of 5x10^5 cells/ml

Cell line	Origin	Media
CHOK1	Chinese hamster ovary	DMEM + 10% FBS + 100U/ml penicillin + 100µg/ml streptomycin
GM01953	Human Lymphoblast, EBV transformed	RPMI 1640 + 10% FBS + 2.2mM glutamine + 100U/ml penicillin + 100µg/ml streptomycin
HeLa	Human Cervical carcinoma	DMEM + 10% FBS + 100U/ml penicillin + 100µg/ml streptomycin

Table 2.1 Name, tissue origin and media requirements of cell lines used

2.2.3 Preparation of GM1953 cells for sucrose density gradient centrifugation

Cells were pelleted by centrifugation at 95x g for 5 minutes and resuspended in fresh medium approximately 24 hours before sucrose density gradient centrifugation was performed. $2.5x10^7$ were resuspended in 50ml fresh medium and grown for 24 hours before harvesting.

2.2.4 Induction of growth arrest by serum starvation in CHOK1 cells

$5x10^6$ cells were seeded onto $10cm^2$ plates then grown for 24 hours in 2ml media. Media was then removed and the cells washed twice with PBS. 2ml media, containing 0.1% serum rather than 10%, was added to the cells, which were then grown for a further 24 hours under these conditions.

2.2.5 FuGENE 6 mediated DNA transfection

24 hours prior to transfection cells were seeded at $0.05x10^6$ cells per well in 1ml media in a 24 well plate. 3µl FuGENE 6 transfection reagent was added to 100µl serum free media and incubated at room temperature for 5 minutes. This solution was then added to 1µg plasmid DNA suspended in 100µl sterile water and incubated at room temperature for a further 15 minutes. The solution was added to cells, dividing it equally between 3 wells. Cells were then grown for 48 hours before harvesting.

2.2.6 Purification of B-CLL cells

White cells were purified from blood samples obtained from patients with B-CLL using Histopaque (Sigma) and following the manufacturer's protocol. Purified B-CLL cells were stored at -80°C. Ethical approval was obtained prior to comencement of the study, and consent from each patient was obtained before samples were taken.

2.2.7 Preparation of B-CLL cells for sucrose density gradient centrifugation

The same purification procedure as described above (2.2.5) was used but cycloheximide was added to blood cells immediately after collection at a concentration of 100µg/ml. Cycloheximide was also added to all subsequent wash solutions at the same concentration.

2.2.8 Purification of tonsil CD19+ B cells

Immediately after removal from healthy volunteers tonsils were added to RPMI 1640 medium, then cut up and passed through a mesh to separate cells into suspension. White cells were purified using Histopaque (Sigma) following the manufacturers protocol and resuspended in RPMI 1640 medium. T cells were then depleted from the white cells using CD3 magnetic beads (Dynabeads®, Dynal Biotech ASA, Oslo Norway) following the manufacturers protocol. Purified B cells were stored at -80°C. Ethical approval was obtained prior to comencement of the study, and consent from each patient was obtained before samples were taken.

2.2.9 Preparation of tonsil CD19+ B cells for sucrose density gradient centrifugation

The same purification procedure as described above 2.2.7) was used but cycloheximide was added to medium and all wash solutions at a final concentration of 100µg/ml.

2.2.10 Primary B cell culture

Purified tonsil CD19+ B cells or B-CLL cells were resuspended at 5x 10^6 cells/ml in 500µl RPMI 1640 medium in a 24 well plate. Cells were grown at 37°C in a humidified atmosphere containing 5% CO_2 for 24 hours before further experimentation was performed.

2.3Bacterial Techniques

2.3.1 Media and supplements

LB Medium: 10g Bacto-tryptone, 5g bacto yeast extract, 10g NaCl dissolved in 1l of deionised water

LB Agar: LB Medium with the addition of 15g Agar

Ampicillin: Stock solution of 100mg/ml ampicillin in sterile deionised water. This was used at a final concentration of 100µg/ml

Bacterial strains: The *E. coli* strains JM109 (Yanisch-Peron et al., 1985) and DH5α (Hanahan, 1983) were used in bacterial manipulations.

2.3.2 Preparation of competent cells for heat shock transformation

A single colony from an LB agar plate was inoculated into 2.5ml of LB medium and incubated overnight at 37°C with shaking. The entire overnight culture was inoculated into 250ml of LB medium supplemented with 20mM $MgSO_4$ and incubated at 37°C until the A_{595} reached 0.4-0.6. Cells were pelleted by centrifugation at 4,500x g for 5 minutes at 4°C using a GSA rotor (Sorvall). The pellet was gently resuspended in 100ml of ice-cold filter sterile TFB1 (30mM KAc, 10mM $CaCl_2$, 50mM $MnCl_2$, 100mM RbCl, 15% glycerol, adjusted to pH 5.8 with 1M acetic acid). After incubating on ice for 5 minutes, the cells were centrifuged at 4,500x g for 5 minutes at 4°C. The pellet was resuspended in 10ml ice-cold filter sterile TFB2 (1mM MOPS, 75mM $CaCl_2$, 10mM RbCl, 15% glycerol, adjusted to pH 6.5 with 1M KOH) and incubated on ice for 1 hour. Finally, cells were rapidly frozen in an isopropanol/dry ice bath in 200µl aliquots and stored at -70°C.

2.3.3 Transformation of competent cells by Heat Shock

Plasmid DNA (5-10ng) or ligation products were added to 50µl competent cells and incubated on ice for 15-20 minutes. The cells were then heat shocked at 42°C for 1 minute. 150µl LB medium was added and the cells were incubated at 37°C with shaking for 30 minutes. They were then spread onto a pre-warmed LB agar plate containing the appropriate antibiotic and incubated at 37°C for 16-20 hours.

2.4 Molecular Biological Techniques

2.4.1 Buffers and solutions

TE: 10mM Tris-HCl pH 7.5, 1mM EDTA

1x TBE: 89mM Tris, 89mM boric acid, 2mM EDTA, pH 8.0

5x DNA loading buffer: 50% sucrose, 100mM EDTA, 0.2% Bromophenol blue, 0.2% Xylene cyanol

Church-Gilbert buffer: 180mM Na_2HPO_4. 70mM NaH_2PO_4, 7% SDS

2.4.2 Plasmids

pRF (described in Stoneley et al 2000b)

2.4.3 Determination of nucleic acid concentration

The concentration of DNA, RNA or oligonucleotides was determined by measuring the optical density of a solution at 260nm.

2.4.4 Ethanol precipitation of nucleic acids

To precipitate DNA or RNA 0.1 volume of 3M sodium acetate pH 5.2 and 2x volume of absolute ethanol were added. Samples were then incubated at -20°C for at least 20 minutes. Nucleic acids could then be pelleted by centrifugation at 12,000g for 15 minutes. Excess salt was removed by washing with 75% ethanol before the pellet was dried and resuspended in deionised water.

2.4.5 Agarose gel electrophoresis

Fragments of DNA or RNA were fractionated according to molecular weight by electrophoresis through agarose gels. 1% agarose gels were made by melting 1g agarose in 100ml 1x TBE. 2µl of 10mg/ml ethidium bromide was added to allow visualisation of nucleic acids and the gel was cast. 5x DNA loading buffer was added to samples, which were then separated at 8V/cm. Nucleic acids were visualised on a uv transilluminator.

2.4.6 Purification of DNA using Wizard® SV Gel and PCR clean-up system (Promega)

The Wizard DNA purification kit (Promega) was used to isolate DNA from agarose gel fragments or to purify DNA fragments, following the manufacturer's protocols.

2.4.7 Oligonucleotides

Oligonucleotides were purchased from Invitrogen and resuspended in sterile water. Sequences of the oligonucleotides used are given in Table 2.2

2.4.8 Polymerase chain reaction (PCR)

Standard PCR reactions were performed in a final volume of 50µl containing 1x PCR buffer (Roche), 0.5-2µg template DNA, 200ng primers, 1mM of each dNTP and 1 unit Taq polymerise (Roche). Reactions were performed in a Techne Genius Thermal Cycler. DNA was initially denatured by heating to 95°C for 5 minutes. The reactions then went through cycles of denaturation, annealing and extension steps, the temperature and time depending on the specific reaction. Denaturation was 95°C for 1 minute, annealing temperatures varied between 50-60°C for 1-2 minutes and extension 72°C for 1-2 minutes. A final extension step was then performed at 72°C for 5 minutes. PCR reactions for GC rich templates were supplemented with 5% Betaine and 5% DMSO.

Oligonucleotide Name	Sequence
ACTINF	CAACCGTGAAAAGATGAC
ACTINR	CCAGACAGCACTGTGTTG
BTG1F	CTGTTCAGGCTTCTCCCAAG
BTG1R`	GAGAGCTGGCTGGTGCTAAC
BTG1UTRF	CGGAGCTGGAATTCCAGCTATTGAGG
BTG1UTRR	TGTAGAAGGGATCCATGGGGGCGGCGTGCG
CSNK2BF	CCCTCACTACCGACAAGCTC
CSNK2BR	GACTGGGCTCTTGAAGTTGC
CSNK2BUTRF	GCTACTAGTTGTGCCCCGCCC
CSNK2BUTRR	AGCTGCCCATGGTCACGTCAG
CCND3F	GAGCTGCTGTGTTGCGAAG
CCND3R	TCTGTAGGAGTGCTGGTCTGG
PABPF	AGGGCAAAAGAATTCACCAATGT
PABPR	GCCACCATGGAAGCAGTCAAAGG
RPS16F	ATGCCGAAGCTTGGCCCGCTGCAG
RPS16R	TTATCGGAATTCTTTCTGGTAGCG
UCP2F	CCCCTACTGCCACTGTGAAG
UCP2R	CAGCTGCTCATAGGTGACGA
UCP2UTRF	CACTGCGAATTCCAGCTGCGC
UCP2UTRR	TGAACCCAACCATGGTGCTGA
WEE1F	GATGTGCGACAGACTCCTCA
WEE1R	GCACTTGTGGTATCCGAGGT

Table 2.2 Sequences of oligonucleotides employed

2.4.9 cDNA synthesis

cDNA was synthesised by reverse transcription from RNA samples. 1-5µg RNA was added to 5µg oligodT primer (Invitrogen) in a volume of 18µl and heated to 70°C for 10 minutes to denature, then allowed to cool and primer annealed by incubation on ice for a further 5

minutes. The remaining components, 1x labelling buffer (Invitrogen), DTT (Invitrogen), 0.2 mM dNTPs and 200 units superscript II (Invitrogen) were added and the reaction incubated at 42°C for 1 hour. The enzyme was then heat inactivated at 95°C for 5 minutes.

2.4.10 Restriction enzyme digestion

To cut DNA at specific sites reaction mixtures were set up containing 10 units each restriction enzyme, 1x restriction enzyme buffer and DNA in a final volume of 30µl. Reactions were then incubated at 37°C for 1-2 hours followed by heat inactivation of enzymes by incubation at 65°C for 10 minutes.

2.4.11 Alkaline phosphatase treatment of DNA

Linearised plasmids were treated with calf intestinal alkaline phosphatase (CIAP) to remove a phosphate group from the 5' ends and prevent self ligation. Following restriction digestion, the restriction enzyme was inactivated by heating the reaction at 65°C for 10 minutes. Dephosphorylation was performed in a final volume of 50µl in 1x restriction enzyme buffer, using 1 unit of CIAP. Reactions were incubated at 37°C for 30 minutes, then DNA purified.

2.4.12 Ligations

Ligations were performed in a total volume of 10µl. Typically 50ng vector DNA was mixed with insert in a 1:3 molar ratio in a reaction containing 1x buffer and 2.5 units T4 DNA ligase. Reactions were incubated at room temperature for 1-16 hours after which transformations were performed with competent *E. coli.*

2.4.13 Small scale preparation of plasmid DNA

A single colony of *E. coli* was inoculated into 5ml of LB media containing ampicillin and incubated at 12-16 hours with shaking. Approximately 1.5ml of the culture was decanted into a labelled tube and the bacteria were pelleted by centrifugation at 12,000x g for 1 minute. The pellet was resuspended in 100µl solution I (25mM Tris-HCl, 10mM EDTA, 50mM glucose, pH 8.0) plus 1µl RNaseA. 200µl solution III (1% SDS, 0.2M NaOH) was then added and the solution mixed gently, followed by incubation on ice for 5 minutes. Then 150µl solution II (7.5M ammonium acetate pH2.4) was added and mixed with a further incubation on ice for 5 minutes. The precipitated matter was pelleted by centrifugation at 12,000x g for 5 minutes and the supernatant transferred to a fresh tube.

Plasmid DNA was precipitated by addition of an equal volume of isopropanol to the supernatant. DNA was then pelleted by centrifugation at 12,000g for 10 minutes and washed with 75% ethanol to remove salt. Purified DNA was resuspended in 30µl TE buffer.

2.4.14 Semi-quantitative RTPCR and southern blotting

cDNA was synthesised, as described in section 2.4.9 RNA samples to use as templates for PCR reactions. Once completed the synthesised cDNA was diluted to 100µl and stored at -20°C until required for further use. Reaction mixtures were set up for the PCR reaction for cDNA samples, in a total volume of 25µl and containing 5µl diluted cDNA, PCR buffer (Roche), 1µM primers, 0.15 units Taq polymerise (Roche) and 1mM $MgCl_2$. 2.5% Betaine and 2.5% DMSO were added if required. Multiple sets of reactions were set up for each cDNA to perform PCR reactions differing in the number of cycles. PCR reactions were performed in a Techne Genius Thermal Cycler. For each reaction mixture, DNA was initially denatured by heating at 95°C for 5 minutes. The reactions then went through cycles of denaturation, annealing and extension steps, the temperature and time depending on the specific reaction. Denaturation was 95°C for 1 minute, annealing temperatures varied between 50-60°C for 1-2 minutes and extension 72°C for 1-2 minutes. The number of PCR cycles performed ranged between 4 and 32, with at least four different cycle lengths performed for 1 experiment. Once each reaction had reached the required number of cycles, it was removed and immediately transferred for storage at -20°C.

5x DNA loading buffer was added to 20µl each PCR product, which were heated to 65°C for 10 minutes. The PCR products were subsequently separated by electrophoresis through a 1% agarose gel, containing 1X TBE buffer. When complete, the gel was soaked in 1.5M/0.5M NaOH for 45 minutes, then 1M Tris, pH7.4/1.5M NaCl for 30 minutes. The DNA was then transferred to Hybond-N membrane (Amersham) using capillary blotting for 18 hours and fixed to the membrane by baking at 80°C for 2 hours before hybridisation.

2.4.15 Synthesis of a radiolabelled DNA probe and hybridisation to immobilised DNA

To prepare a random-primed radiolabelled probe, 30ng of DNA template, in 15µl deionised water was heated at 95°C for 5 minutes then immediately cooled on ice. A reaction was then set up containing 1x labelling buffer (Promega), 0.4mg/ml BSA, 0.02mM dATP, dGTP and dTTP, 20µCi [α-^{32}P]dCTP (3000 Ci/mmol) and 5 units Klenow DNA

polymerise (Roche) in a total volume of 25µl and incubated at room temperature for 2 hours. Unincorporated nucleotides were removed by passing the probe through a Sephadex G-50 column. The RNA/DNA bound to the membrane was pre-hybridised with 10ml of Church-Gilbert buffer for 30-60 minutes. The random-primed DNA probe was added directly following denaturation at 95°C for 5 minutes. The membrane was then hybridised at 65°C for 16-24 hours. Following hybridisation the membrane was washed twice with 2x SSC + 0.1% SDS solution for 15 minutes, then twice with 0.2x SSC + 0.1% SDS solution for 30 minutes. Further washes with 0.2x SSC + 0.1% SDS solution were performed if required. Excess moisture was then removed and radiolabelled probe detected by phosphoimager analysis.

2.5 RNA Techniques

2.5.1 Buffers and solutions

10% sucrose gradient solution: 10% w/v sucrose, 15mM Tris pH 7.5, 15mM $MgCl_2$, 300mM NaCl, 1mM DTT, 0.5mg/ml heparin

18% sucrose gradient solution: 18% w/v sucrose, 15mM Tris pH 7.5, 15mM $MgCl_2$, 300mM NaCl, 1mM DTT, 0.5mg/ml heparin

26% sucrose gradient solution: 26% w/v sucrose, 15mM Tris pH 7.5, 15mM $MgCl_2$, 300mM NaCl, 1mM DTT, 0.5mg/ml heparin

34% sucrose gradient solution: 34% w/v sucrose, 15mM Tris pH 7.5, 15mM $MgCl_2$, 300mM NaCl, 1mM DTT, 0.5mg/ml heparin

42% sucrose gradient solution: 42% w/v 15mM Tris pH 7.5, 15mM $MgCl_2$, 300mM NaCl, 1mM DTT, 0.5mg/ml heparin

50% sucrose gradient solution: 50% w/v sucrose, 15mM Tris pH 7.5, 15mM $MgCl_2$, 300mM NaCl, 1mM DTT, 0.5mg/ml heparin

60% sucrose gradient solution: 60% w/v sucrose, 15mM Tris pH 7.5, 15mM $MgCl_2$, 300mM NaCl, 1mM DTT

65% sucrose gradient solution: 65% w/v sucrose, 15mM Tris pH 7.5, 15mM $MgCl_2$, 300mM NaCl, 0.5% w/v bromophenol blue

Polysome lysis buffer: 15mM Tris pH 7.5, 15mM $MgCl_2$, 300mM NaCl, 1% Triton X-100, 5mM DTT, 100µg/ml cycloheximide, 1mg/ml heparin

5x MOPS buffer: 0.1M MOPS pH 7, 40mM NaAc, 5mM EDTA

20x SSC: 3M NaCl, 300mM tri-sodium citrate

2.5.2 Isolation of total cellular RNA

Total RNA was isolated using TRI reagent (Sigma). Suspension cells were pelleted by centrifugation at 550x g for 10 minutes and washed with PBS, then TRI reagent added to lyse the cells. Adherent cells were washed with PBS and lysed with TRI reagent *in situ* and lysates removed with a cell scraper and transferred to a fresh tube. 1ml TRI reagent was used for every 5-10x10^6 cells. 200µl chloroform was added per ml TRI reagent and the mixture vortexed then left to stand for at least 30 seconds before centrifugation at 12,000x g for 15 minutes. The upper aqueous phase was transferred to a fresh tube and isopropanol added. The sample was then incubated at -80°C for 30 minutes, followed by centrifugation at 12,000x g for 15 minutes to pellet precipitated RNA. The pellet was then washed in 75% ethanol to remove salt before drying and resuspension in deionised water.

2.5.3 Preparation of sucrose density gradients

Sucrose density gradients were prepared in 12ml polyallomer centrifuge tubes (Sorvall, Newtown, Connecticut, USA). 500µl 60% sucrose solution was added to a gradient tube and frozen on dry ice. 1.6ml 50% sucrose solution was then added to the tube and frozen using the same procedure. 1.6ml additions of 42%, 34%, 26%, 18% and 10% sucrose solutions were also added allowing each layer to freeze before addition of the next. Sucrose gradients were stored at -20°C and allowed to thaw before use.

2.5.4 Sucrose density gradient centrifugation

GM1953 cells were resuspended in fresh medium 24 hours prior to harvesting as described in 2.2.3. Cycloheximide was added to cells at a final concentration of 100µg/ml and incubated for 10 minutes at 37°C. The cells were washed twice with ice cold PBS containing 100µg/ml cycloheximide and then pelleted by centrifugation at 550x g for 10 minutes at 4°C. Primary cells were prepared for sucrose density gradient centrifugation as described in 2.2.6 and 2.2.8 and thawed immediately before use. Pellets were lysed in 400µl polysome lysis buffer, 1mg/ml heparin, 100µg/ml cycloheximide and 150U/ml Prime RNase inhibitor (Helena Biosciences). For GM1953 cells, 2.5x10^7 cells were contained in each pellet. For primary cells, a maximum of 5x10^8 cells were in each pellet. The lysate was centrifuged at 12,000x g for 5 minutes at 4°C. The supernatant was then gently loaded onto a sucrose density gradient. The gradients were centrifuged at 153,000x g for 2 hours at 4°C and stored on ice until analysed.

65% sucrose solution was used to force the gradient solution through a Minipuls 2 (Anachem, Luton, Bedfordshire) and absorbance measured at 254nm by a UA-6 UV/VIS detector (ISCO c/o Presearch, Hitchin, Herts). The gradient solution was collected in 1ml fractions using a Model 2110 fraction collector (Bio-Rad, Hemel Hempstead, Hertfordshire) and each immediately transferred into 4ml 7.7M guanidine hydrochloride and 4ml absolute ethanol.

2.5.5 Isolation of RNA from sucrose gradient fractions

RNA was precipitated from each 1ml sucrose density gradient fraction by addition of 4ml 7.7M guanidine hydrochloride and 4ml absolute ethanol and incubation at -20°C for 12 hours. RNA was pelleted by centrifugation at 3,220x g for 45 minutes at 4°C. The supernatant was then discarded and the RNA pellet washed in 75% ethanol. Following centrifugation at 12,000x g for 10 minutes at 4°C, RNA was ethanol precipitated at –20°C for 2 hours and then pelleted by centrifugation at 12,000x g for 15 minutes at 4°C. The pellets were then washed in 75% ethanol and resuspended in deionised water.

2.5.6 Lithium chloride precipitation of RNA

To remove all traces of heparin, RNA from sucrose gradients was lithium chloride precipitated. RNA resuspended in deionised water was mixed with an equal volume of 5M lithium chloride. RNA was precipitated by incubation at -20°C for 30 minutes and then pelleted by centrifugation at 12,000x g for 15 minutes. The pellet was then washed with 75% ethanol to remove salt and resuspended in deionised water.

2.5.7 Denaturing RNA agarose gel electrophoresis and Northern blotting

Samples of RNA were denatured by addition of 1x gel running buffer (Sigma) and incubation at 65°C for 10 minutes. The RNA was then fractionated by electrophoresis through a 1% agarose gel containing 1x MOPS buffer and 17.5% v/v formaldehyde. The gel was submerged in 1x gel buffer and run at 120V for 2-3 hours. After, electrophoresis was complete, RNA was visualised using a UV transilluminator and photographed for later reference. The gel was then washed in deionised water before being soaked in 0.05M NaOH for 15 minutes, then 20x SSC for 30 minutes. The RNA was then transferred from the gel to Hybond-N membrane (Amersham) using capillary blotting for 18 hours and fixed to the membrane by baking at 80°C for 2 hours before hybridisation.

2.5.8 Synthesis of a radiolabelled DNA probe and hybridisation to immobilised RNA

Random-primed radiolabelled probes were synthesised as described in section 2.4.15

2.5.9 Stripping of radiolabelled DNA probe from immobilised RNA

In order to hybridise a Northern membrane with a different radiolabelled DNA probe, the hybridised probe was stripped by adding boiling 0.5% SDS solution and shaking at 100rpm for 30 minutes. This process was repeated if required.

2.6 Biochemical Techniques

2.6.1 Buffers and solutions

1x SDS sample buffer: 50mM Tris pH 6.8, 10% glycerol, 4% SDS, 0.1% bromophenol blue, 10% ß-mercaptoethanol, 1mM EDTA

SDS-PAGE resolving buffer: 1.5M Tris, 0.24% TEMED, 1% SDS pH8.8

SDS-PAGE stacking buffer: 0.25M Tris, 0.12% TEMED, 0.2% SDS pH 6.8

1x SDS running buffer: 25mM Tris, 192mM glycine, 0.1% SDS pH8.3

TBST (Tris buffered saline, Tween): 10mM Tris pH 8.0, 0.9% NaCl, 0.1% Tween

Cell lysis buffer: 50mM MOPs pH 7.2, 50mM NaCl, 50mM ßG(P), 2mM EGTA, 5mM EDTA, 10% NaF, 1% Benzamadine, 0.05% BME, 1% Triton X-100

2.6.2 Preparation of cell lysates from transfected cells

After transfection of cells, the medium was aspirated from cells were then washed twice with PBS. The cells were lysed by addition of 1x Passive lysis buffer (Promega). The cells were subject to one freeze-thaw cycle at -20°C to increase lysis efficiency.

2.6.3 Luciferase assays

The activity of *Renilla* and firefly luciferases in lysates of cells transfected with dicistronic luciferase plasmids was measured using a Dual luciferase reporter assay system (Promega). 5µl of lysate prepared using passive lysis buffer was used for each assay. Assays were performed according to the manufacturer's protocol, except that only 25µl of each reagent was used. Light emission was measured over 10 seconds using an Optocomp I luminometer (MGM Instruments).

2.6.4 Determination of protein concentration by Bradford assay

Stock BSA (1mg/ml) was diluted to concentrations of 1-32µg/µl. Bradford reagent (Pierce and Warriner) was added to the BSA dilutions plus cell extract samples according to the manufacturer's protocol and the absorbance at 595nm was measured. Protein concentration in cell extracts was determined using a standard curve.

2.6.5 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

1x SDS sample buffer was added to protein extracts or whole cell samples to denature protein. For whole cell samples, DNA was sheered by sonication. Samples were heated to 95°C for 5 minutes prior to loading. SDS-polyacrylamide gels were prepared as detailed in Table 2.3 and polymerised by the addition of ammonium persulphate (APS) solution. Gels were run in a Bio-Rad Protean II system in SDS running buffer according to standard procedures. Typically, gels were run at a constant voltage of 150V until the Bromophenol blue dye front reached the bottom of the gel.

% gel	Water	Resolving/stacking Buffer	30%:0.8% acrylamide: bisacrylamide solution
10	2.1ml	1.25ml	1.67ml
12.5	1.7ml	1.25ml	2.00ml
Stacking gel	0.9ml	1.25ml	0.33ml

Table 2.3 Composition of SDS-PAGE gels

2.6.6 Coomassie staining of SDS-polyacrylamide gels

Gels were stained in a coomassie staining solution for 30 minutes at room temperature and subsequently destained in destaining solution for 3-5 hours. Gels were then incubated in deionised water for 30 minutes to remove acetic acid before drying.

2.6.7 Transfer of proteins onto nitrocellulose membranes

Cell extracts separated by SDS-PAGE were transferred onto nitrocellulose (Schleider and Schuell, Dassel, Germany) by semi-dry blotting in transfer buffer (50mM Tris, 192mM glycine, 20% methanol) for between 30 and 90 minutes at 10V. Protein transfer was visualised temporarily with Ponceau-S solution (0.5% w/v in 5% w/v trichloroacetic acid [TCA]).

2.6.8 Western blotting

Proteins immobilized on to nitrocellulose after SDS-PAGE were detected immunologically. Nitrocellulose membranes were incubated in a 5% dried milk solution in TBST for 1 hour at room temperature to block non-specific binding sites. Membranes were then incubated in 5-10ml of primary antibody diluted appropriately in 5% milk TBST for 12-16 hours at 4°C with constant agitation. After three 10 minute washes in TBST, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies to mouse IgG (Dako A/S, Denmark), goat IgG (Dako A/S, Denmark) or rabbit IgG (Sigma), diluted to 1:2000 (for mouse or goat) or 1:10,000 (for rabbit) in 5% milk TBST, for 1 hour at room temperature with constant agitation. Three 10 minute washes in TBST solution were carried out and protein-antibody complexes were detected using an enhanced chemiluminescence (ECL) technique. For this, 1ml Luminol solution (50mg Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) in 0.1M Tris-HCl pH6.8), 10µl Enhancer (11 mg para-coumaric acid in 10ml DMSO) and 3.1µl 3% hydrogen peroxide were mixed and incubated on the membrane for 60 seconds. Chemiluminescence was visualised by exposing the membrane to X-ray film for periods of between 10 seconds and 30 minutes.

2.6.9 Stripping and reprobing of western blots

Nitrocellulose membranes were stripped of existing protein-antibody interactions by incubation in a solution of 100mM ß-mercaptoethanol, 2% SDS and 62.5mM Tris-HCl pH 6.7 for 10 minutes at 50°C. Membranes were then washed in TBST and re-probed with a different primary antibody as described above.

2.6.10 Determination of translation rates using ^{35}S methionine incorporation into protein

10µCi ^{35}S methionine (1000 Ci/mmol) was added to 10cm^2 plates of cells for 20 minutes and cells incubated at 37°C for 20 minutes. Media was then aspirated from adherent cells, which were then washed twice with PBS. Cells were lysed by the addition of 200µl cell lysis buffer and lysates removed from wells with a cell scraper then transferred to a fresh eppendorf tube. Incorporation of ^{35}S methionine into protein was determined for 75µg protein. Protein was precipitated by addition of 1ml 10% TCA and incubation on ice for 10 minutes. The precipitated protein was then filtered onto Whatman glass fibre filter circles, washed thoroughly with 10% TCA, 70% IMS, then butanol and air died. Dried papers were

placed in scintillation vials with scintillation fluid and methionine incorporation measured by counting for 1 minute using a scintillation counting.

2.7 Microarray Techniques

2.7.1 Buffers and solutions

TE: 10mM Tris-HCl pH 7.5, 1mM EDTA

50x denhardts: 1% w/v Ficoll (Type 400, Pharmacia), 1% w/v polyvinylpyrrolidone, 1% w/v BSA

Hybridisation buffer: 70% deionised formamide, 3.5x denhardts, 0.7% SDS

20x SSPE: 3M NaCl, 1mM NaH_2PO_4, 20mM EDTA. pH to 7.4

50x microarray dNTP stock: 25mM dATP, 25mM dCTP, 25mM dGTP, 15mM dTTP, 10mM aminoacyl-dUTP

2.7.2 Preparation of microarray slides for hybridisation

Microarray slides were provided by Dr Tim Gant (University of Leicester, UK). DNA was fixed to the slides by baking at 80°C for 2 hours in a Peltier PTC-225 thermal cycler (MJ Research, Waltham, Massachusetts). The slides were then washed twice for 1 minute in 0.2% SDS solution and twice for 1 minute in deionised water, before drying by centrifugation at 65x g for 2 minutes. DNA was then denatured by heating to 100°C for 2 minutes in the thermal cycler. The slides were briefly rinsed in deionised water and centrifuged again at 65x g for 2 minutes to dry. Slides were stored in the dark.

2.7.3 Preparation of fluorescently labelled dyes for coupling reactions

Cy3 and cy5 mono-reactive fluorescent dyes (Amersham) were resuspended in 20µl DMSO and aliquoted into 2µl fractions. The dyes were dried by centrifugation in a DNA SpeedVac 120 (Savant, New York) for 1 hour and stored at -20°C in the dark.

2.7.4 Preparation of cDNA by reverse transcription (RT)

RT reactions were performed to prepare cDNA for microarray hybridisation from total RNA or messenger RNA. 5µg oligo-dT was added to RNA and the volume made up to 18µl with deionised water. The RNA was heated to 70°C for 10 minutes and cooled on ice for 5 minutes. The reaction mix was then made up to 30µl containing 1x Superscript™ II first strand RT buffer (Invitrogen), 1x microarray dNTP stock, 10mM DTT and 400 units

of Superscript™ II reverse transcriptase (Invitrogen). Reactions were incubated for 1 hour. A further 200 units Superscript™ II were then added and the reaction incubated for a further 1 hour.

2.7.5 Hydrolysis and cleanup of cDNA

Once cDNA synthesis was complete, RNA was degraded by addition of NaOH to the RT reaction to a final concentration of 0.03M and the reaction incubated at 70°C for 10 minutes. To neutralise the reaction HCl was added to a concentration equivalent to that of NaOH. Deionised water was then added to the RT reaction making the volume up to 500µl, loaded onto a Microcon-30 filter (Amicon, Millipore, Watford, Hertfordshire) and centrifuged at 8000x g for 7 minutes. The flow-through was discarded and the filter washed twice by the addition of 450µl deionised water and centrifuged at 8000x g for 7 minutes. The cDNA was then concentrated to 10µl or less by centrifugation at 8000x g for 30 seconds. If necessary, the volume of cDNA was increased to 10µl using deionised water.

2.7.6 Coupling of cDNA and fluorescently labelled dyes

Sodium bicarbonate pH 9.0 was added to the cDNA sample to give a final concentration of 0.05M. The cDNA sample was then added to a cy3 or cy5 pellet (prepared as described in section 2.6.3) and mixed to resuspended the dye. The sample was then incubated at room temperature in the dark for 1 hour, mixing every 15 minutes.

2.7.7 Quenching and cleanup of labelled cDNA

Once the dye coupling reaction was complete, hydroxylamine was added to give a final concentration of 1.2M and the reaction incubated at room temperature in the dark for 15 minutes. The cy3 and cy5 reactions required for a microarray experiment were then combined, diluted with 70µl deionised water and 500µl buffer PB (Qiagen) and then applied to a QIAquick® spin column (Qiagen). The diluted reaction was centrifuged at 8,000x g for 30-60 seconds and the flow through discarded. The column was washed by addition of 750µl buffer PE (Qiagen) and centrifugation at 8,000x g for 30-60 seconds and the flow-through discarded. The column was then dried by centrifugation at 8,000x g for 1 minute. To elute the cDNA, 30µl buffer EB (Qiagen) was added to the column and incubated at room temperature for 3 minutes before centrifugation at 8,000x g for 1 minute. This was then repeated with an additional 30µl buffer EB to elute the cDNA to give a final volume of 60µl.

2.7.8 Hybridisation of labelled cDNA to microarray slides

20µg human Cot-1 DNA (Invitrogen), 20µg polyA RNA (Sigma) and 20µg yeast tRNA (Sigma) were added to the labelled cDNA. TE was added to a final volume of 500µl and applied to a Microcon-30 spin column. The cDNA was concentrated to 10µl by centrifugation at 8,000x g and eluted by inversion of the spin column and centrifugation at 8000x g for 30 seconds. The concentrated cDNA was then dried using a DNA speedVac 120 centrifuge (Savant, New York) for 10-15 minutes and resuspended in hybridisation buffer and 3x SSPE in a total volume of 20µl. The cDNA was then heated to 100°C for 2 minutes and incubated at 42°C for 30-60 minutes. The cDNA was applied to the microarray slide and spread over the grid by addition of a coverslip. 5µl spots of 3x SSC were placed around the coverslip and the slide placed in a HybChamber™ (Gene Machines, c/o Genomic solutions, Huntingdon, Cambridgeshire). Slides were incubated at 42°C for 14-18 hours.

2.7.9 Post-hybridisation washing of microarray slides

Following hybridisation, slides were removed from the hybridisation chamber and immediately immersed in 2x SSC, 0.1% SDS solution. The slides were left for 30-60 seconds to allow the coverslips to fall off then briefly agitated before being shaken at 90 rpm for 5 minutes. The slides were then transferred into 1x SSC solution and shaken at 90rpm for another 5 minutes. Finally, the slides were transferred to a 0.2x SSC solution and shaken again for 5 minutes, then dried by centrifugation at 65x g for 2 minutes.

2.7.10 Scanning of microarray slides

Microarray slides were scanned using a GenePix 4000A microarray scanner and GenePix Pro 3.0 software (Axon Instruments, Union City, California).

2.7.11 Analysis of microarray data

GenePix Pro 3.0 was used to quantify fluorescence intensities for individual spots on the microarray. The data obtained was then normalised and subjected to T-test statistical analysis using an MRC Toxicology Unit program, Normalise and T-test Gene Expression Data 1.1.0. For comparison of data between two cell types a T-test with unequal variance was used. Manipulation of the data throughout analysis was achieved using Microsoft Excel, GeneSeeker, Clone Mapper 2.1.5 and clone information on the MRC Toxicology website (http://www.le.ac.uk/mrctox/microarray_lab/). Another MRC Toxicology unit

program, GO_parser_1, was subsequently used to identify genes according to GO terms relating to function and cellular location information. Data was visualised using TIGR MeV 2.2 (Saeed et al 2003, http://www.tigr.org/software/tm4/menu/TM4_Biotechniques_2003.pdf).

Chapter 3

Determination of translation status in B-CLL

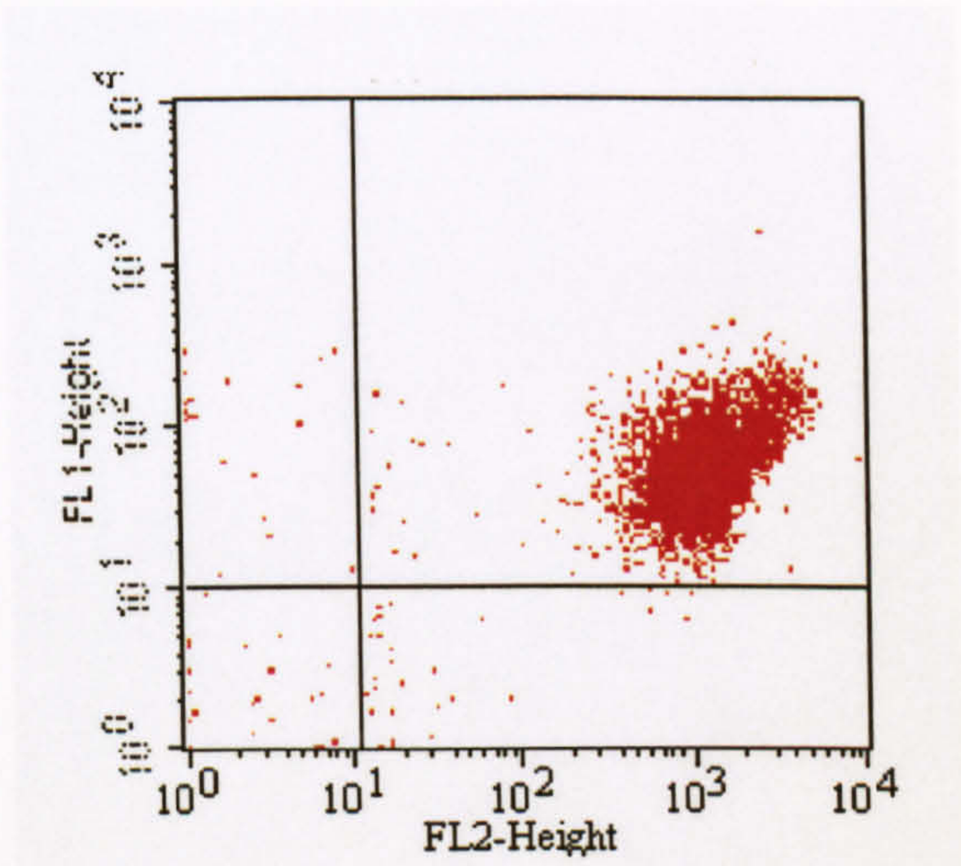
3.1 Introduction

Translation regulation has become recognised as an important step in the control of gene expression with respect to cancer, as discussed in section 1.5. In particular, many genes involved in cell proliferation and growth or apoptosis are translationally regulated, processes often deregulated in tumorigenesis. Therefore analysis of translation in B-CLL cells may provide a useful insight into the biology of the disease. The data obtained suggested that a reduced level of translation was occurring in B-CLL cells compared to GM1953 cells and tonsil CD19+ B cells. A subgroup of patients was also identified, which may be defined by a difference in protein levels of some translation regulatory factors.

3.2 Purification of B-CLL and tonsil CD19+ B cells

To isolate primary B-CLL cells from patient samples, 30ml blood samples were obtained, adding cycloheximide immediately to 'fix' ribosomes to mRNA molecules. White cells were isolated using Histopaque, and subsequent staining with the cell surface markers CD19 and CD5 followed by flow cytometric analysis (FACs), revealed samples were approximately 95% B-CLL cells so no further purification was required (Figure 3.1).

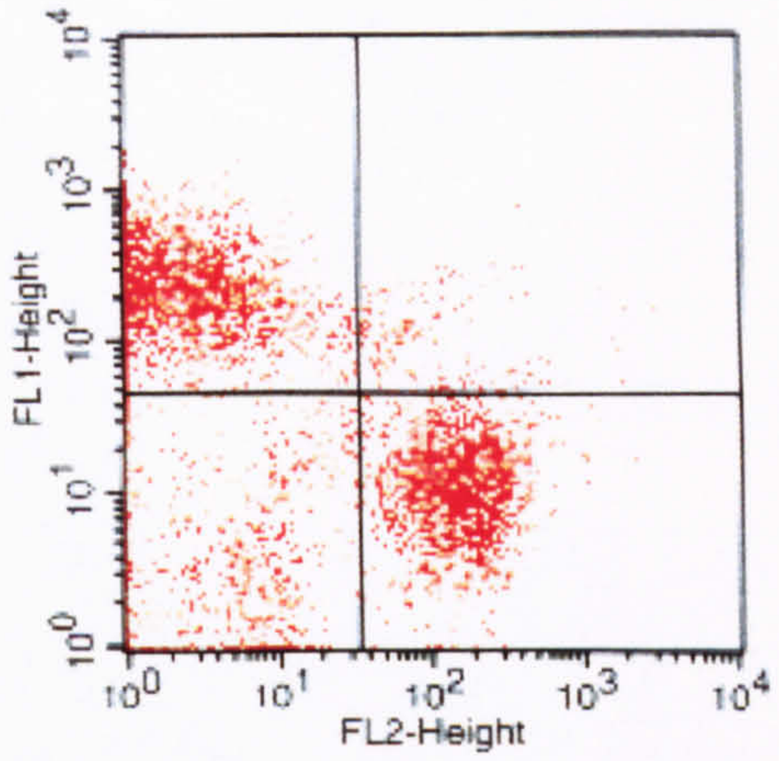
Primary CD19+ B cells were used as a control and were isolated from tonsil samples donated by healthy volunteers. Again, ribosomes were 'fixed' to mRNA molecules with cycloheximide and white cells isolated from samples using Histopaque. Staining of cells for CD19 and CD3 then FACS analysis, to determine the proportion of B and T cells in the samples, revealed samples to contain approximately 45% B cells and 30% T cells (Figure 3.2A). T cells were subsequently depleted using CD3 magnetic beads, and staining and FACS analysis revealed almost 100% removal of T cells was achieved. This increased the purity of CD19+ B cells to approximately 80% (Figure 3.3A). Viability, determined by propidium iodide staining was also observed to be approximately 80% (Figure 3.3B). (All FACs analysis was performed by Dr Marion MacFarlane).



CD19/CD5 + 98.54%

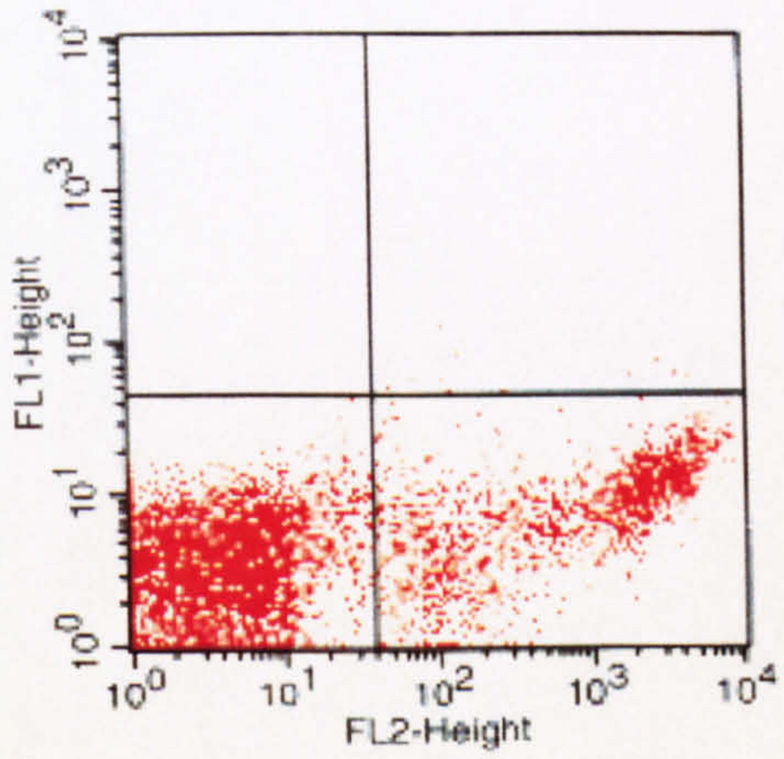
Figure 3.1 Composition of B-CLL patient samples after purification of white cells. After purification cells were stained with CD19 and CD5. The number of stained cells was determined by flow cytometric analysis. The data shown is representative for all patients.

A:



CD19+ 58.26%
CD3+ 27.36%

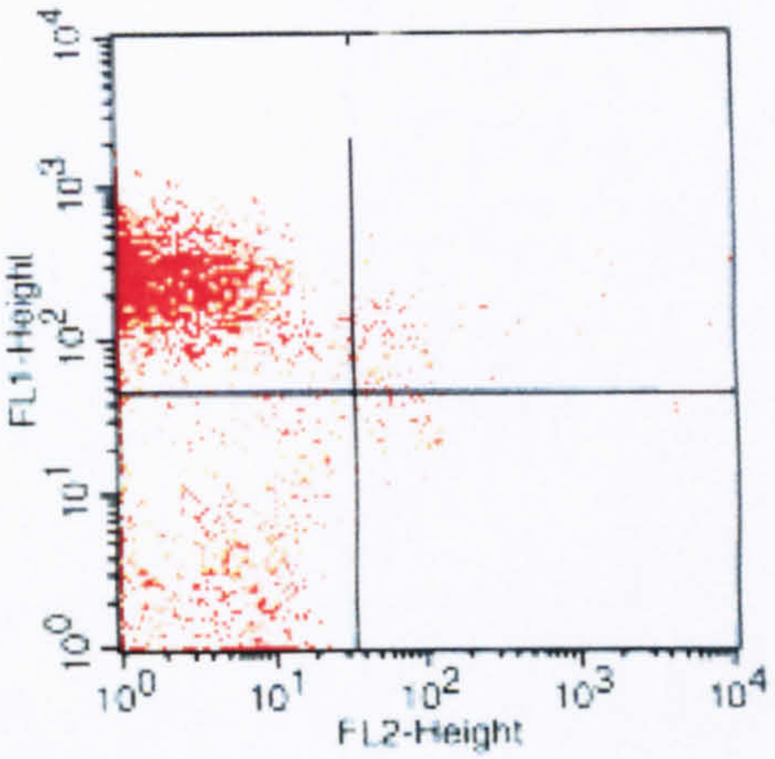
B:



PI+ 28.79%
PI– 70.96%

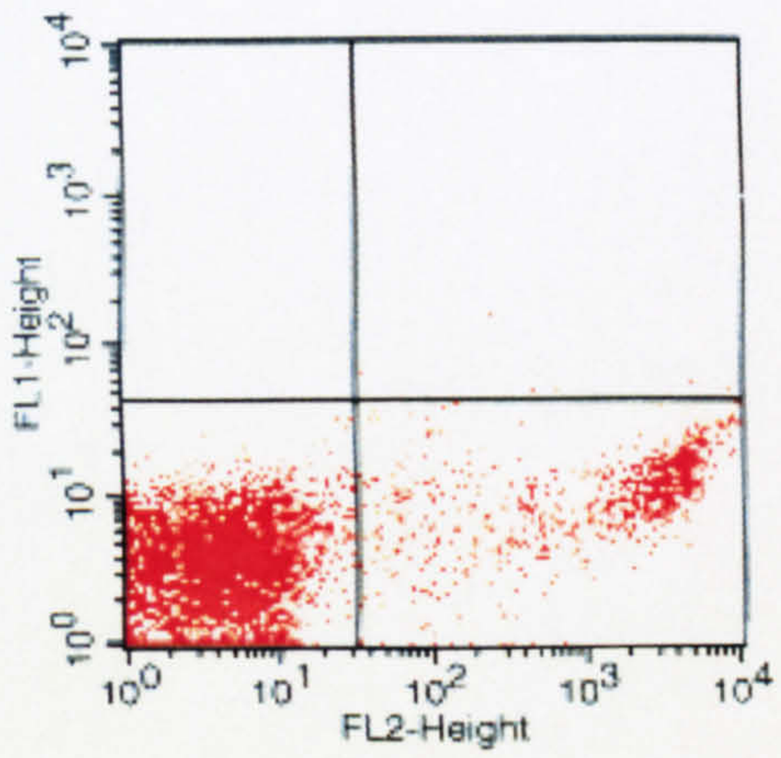
Figure 3.2 Composition of tonsil samples after purification of white cells. After purification cells were stained for CD19 and CD3 markers (A), or with propidium iodide (B). The number of stained cells was determined by flow cytometric analysis. The data shown is representative for all samples.

A:



CD19+ 84.65%
CD3+ 0.92%

B:



PI+ 17.35%
PI– 82.52%

Figure 3.3 Composition of tonsil samples after CD3+ cell depletion
After purification cells were stained for CD19 and CD3 markers (A), or with propidium iodide (B). The number of stained cells was determined by flow cytometric analysis. The data shown is representative for all samples.

3.3 Determination of translation factor status in B-CLL

The levels of translation factors in B-CLL cells were determined using western blot analysis. B-CLL cell and tonsil CD19+ B cell samples were separated by SDS-PAGE, then immunoblotted and probed with the antibodies shown (Figure 3.4 and Figure 3.5). eIF2α levels appeared to be similar in B-CLL samples compared to tonsil CD19+ B cells, although may be slightly lower in two of the patients (patients 1 and 4) (Figure 3.4). Interestingly, eIF4E and 4EBP1 levels were reduced in the same two patients (Figure 3.4). eIF4E is thought to be a limiting factor for the formation of 4F complexes therefore theoretically, lower levels would reduce the amount of active 4F formed and could cause a decrease in the amount of translation initiation occurring in B-CLL cells. However, a reduction in 4EBP1 protein levels would reduce the amount of eIF4E sequestered in an inhibitory complex with this protein. The variation of translation factors between B-CLL patients observed may define two disease subgroups identified in a recent study (Dürig et al., 2003). Microarray analysis identified two subgroups for B-CLL, which differed in the level of transcription for a number of ribosome and translation associated genes. Protein levels were not analysed in this study but the differences in levels of eIF4E and 4EBP1 observed may correlate with this data. eEF2 levels were also observed to vary between the B-CLL patients although most were the same as or slightly lower than levels in tonsil CD19+ B cells and did not show the same pattern of expression as eIF2α, eIF4E or 4EBP1 (Figure 3.5). eEF2 kinase and p70S6 kinase levels were equivalent in tonsil and B-CLL samples (Figure 3.5).

3.4 Analysis of polysome-subpolysome distribution of mRNAs

To further analyse translation in B-CLL cells, the polysome-subpolysome distribution was recorded. This type of analysis can be used to identify which messages are polysomal (multiple ribosomes attached) and assumed to be translated, or subpolysomal and assumed to be untranslated. Polysome-associated messages were separated from subpolysome messages using 10-50% sucrose density gradients. Cell lysate was layered upon a sucrose density gradient, which was then centrifuged. The position a message sediments in the gradient depends upon the number of ribosomes attached (Figure 3.6). A polysome profile is produced by measuring absorbance at 254nm across the gradient. A profile was recorded for the B cell line GM1953 with sedimentation from left to right (Figure 3.7). The peaks for the 40S and 60S ribosome are highlighted. Sometimes a peak for messages associated with one ribosome (monosomes) is seen but this can become merged with the 60S peak as was

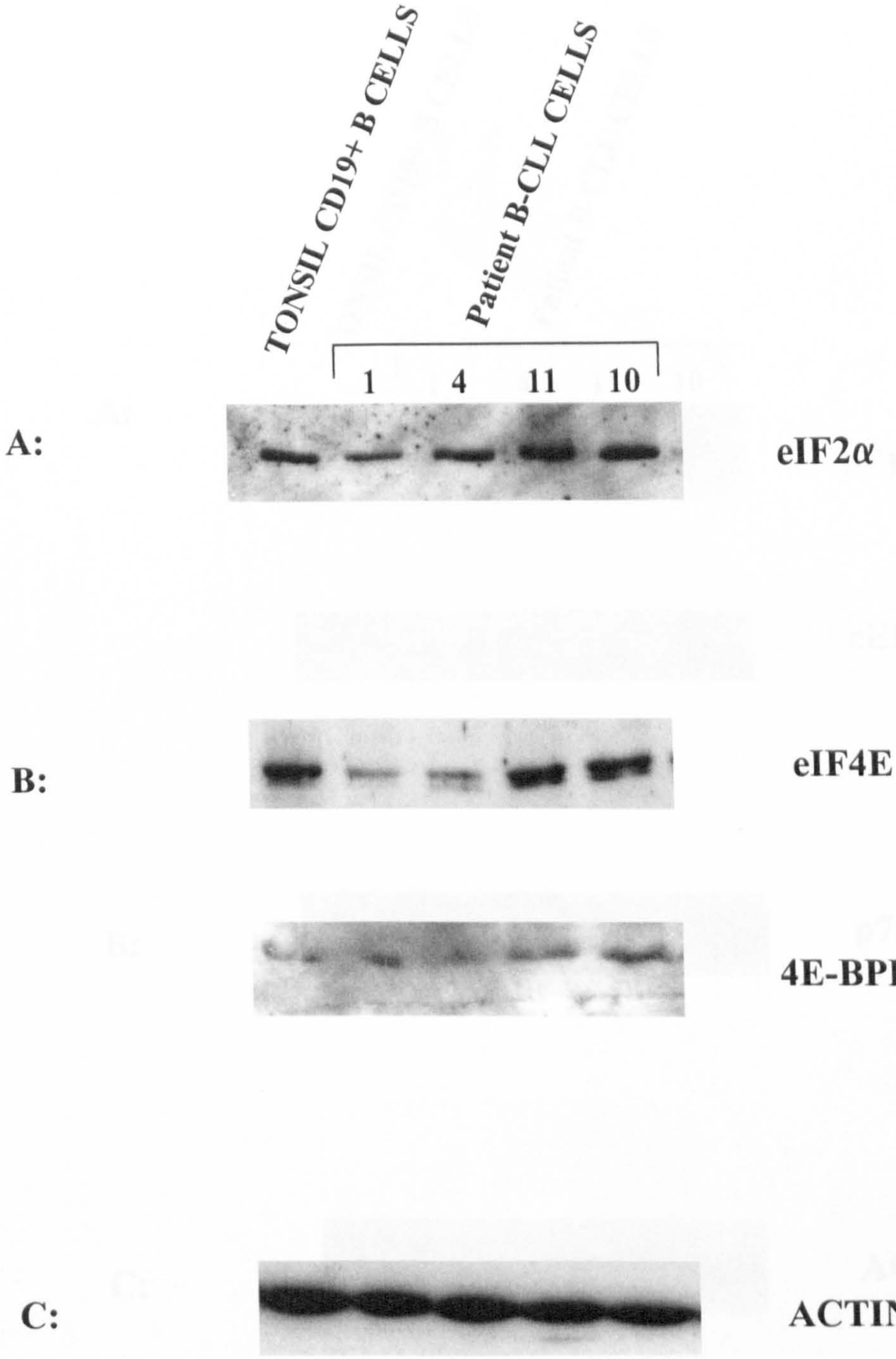


Figure 3.4 Western analysis of translation factors in B-CLL. Cell lysates were produced for tonsil CD19+ B cells and B-CLL cells (patients 1, 4, 11 and 10) and separated by SDS PAGE. The gels were then immunobloted and probed for with specific antibodies to eIF2α (A), eIF4E and 4EBP-I (B) and actin (C). Variation in the levels of eIF2α and eIF4E were observed for B-CLL patients. Each Western was performed once.

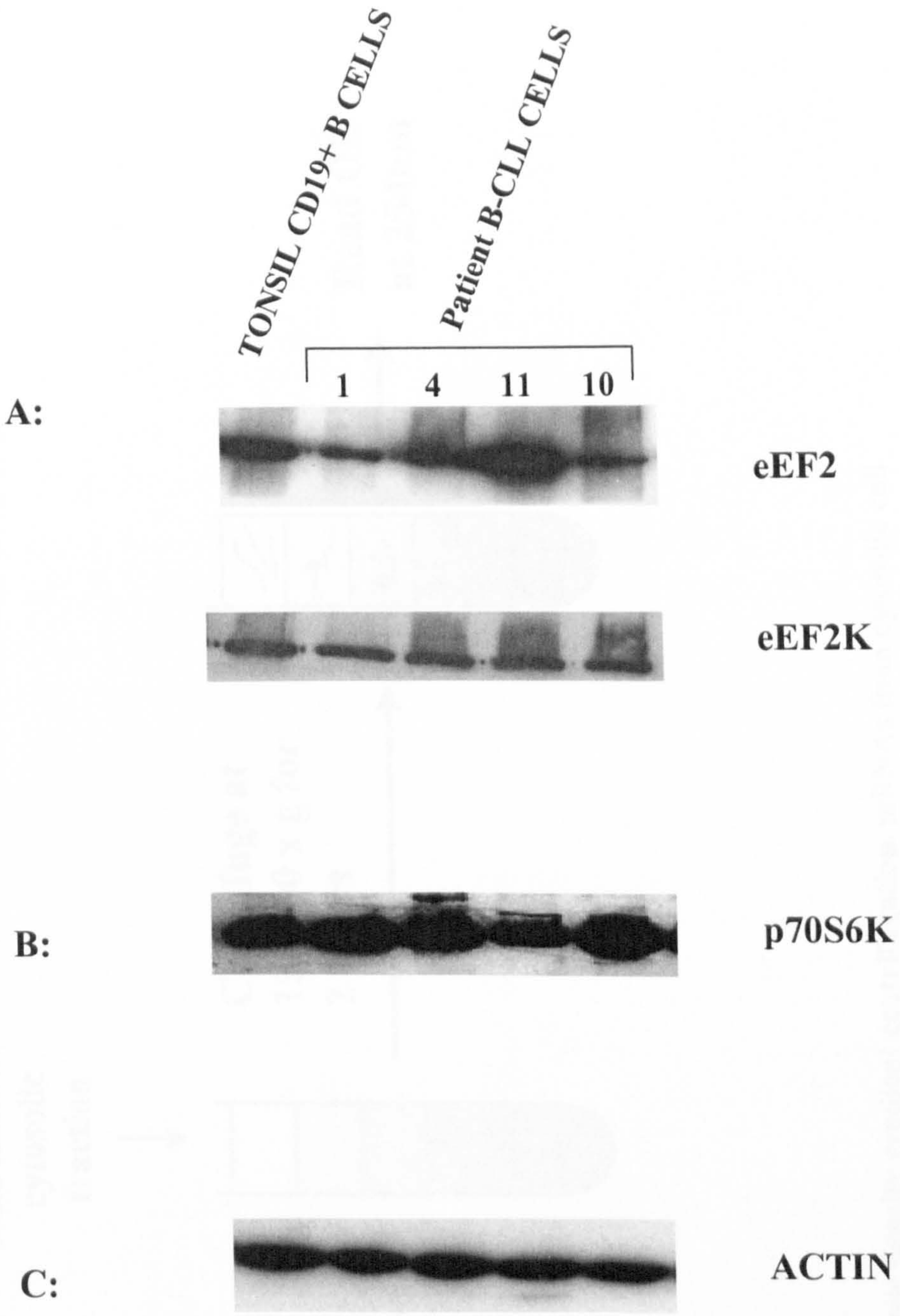


Figure 3.5 Western analysis of translation factors in B-CLL. Cell lysates were produced for tonsil CD19+ B cells and B-CLL cells (patients 1, 4, 11 and 10) and separated by SDS PAGE. The gels were then immunobloted and probed for with specific antibodies to eEF2, eEF2K (A), p70S6K (B) and actin (C). Each Western was performed once.

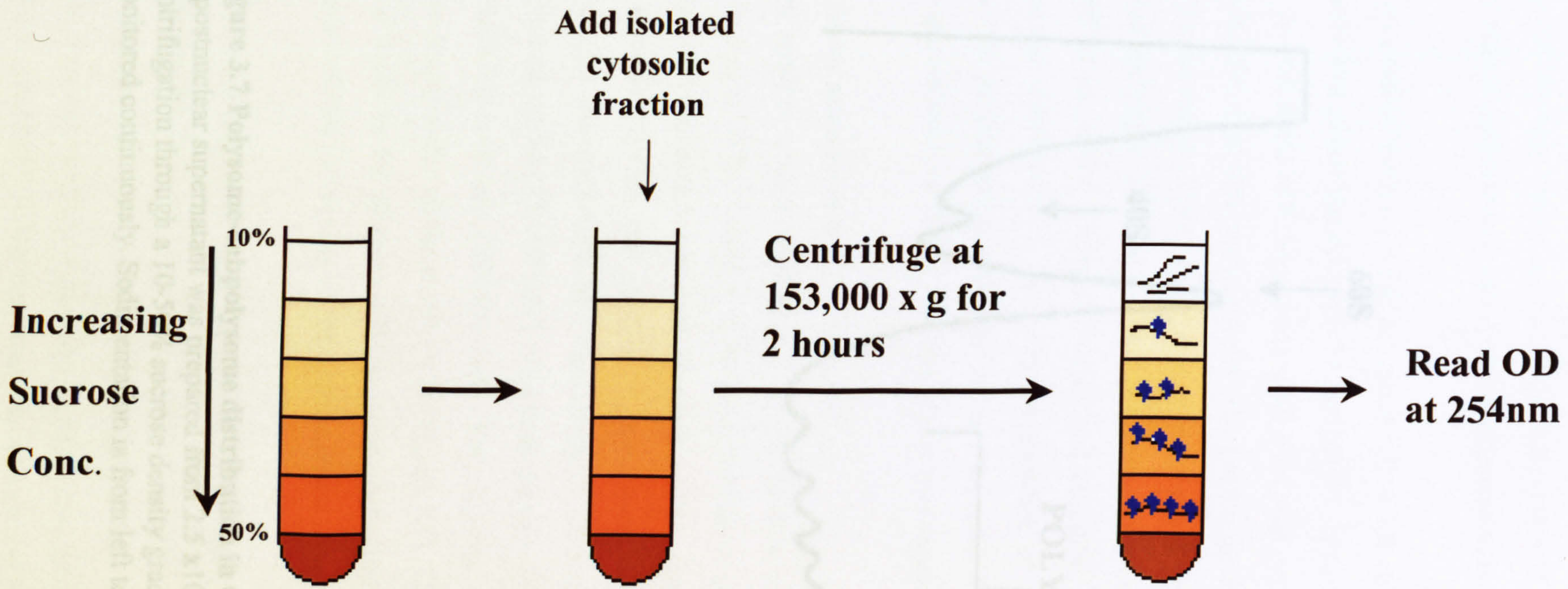


Figure 3.6 Sucrose density gradient centrifugation. mRNAs from cytosolic cell fractions were separated, according to the number of ribosomes attached, by centrifugation in sucrose density gradients.

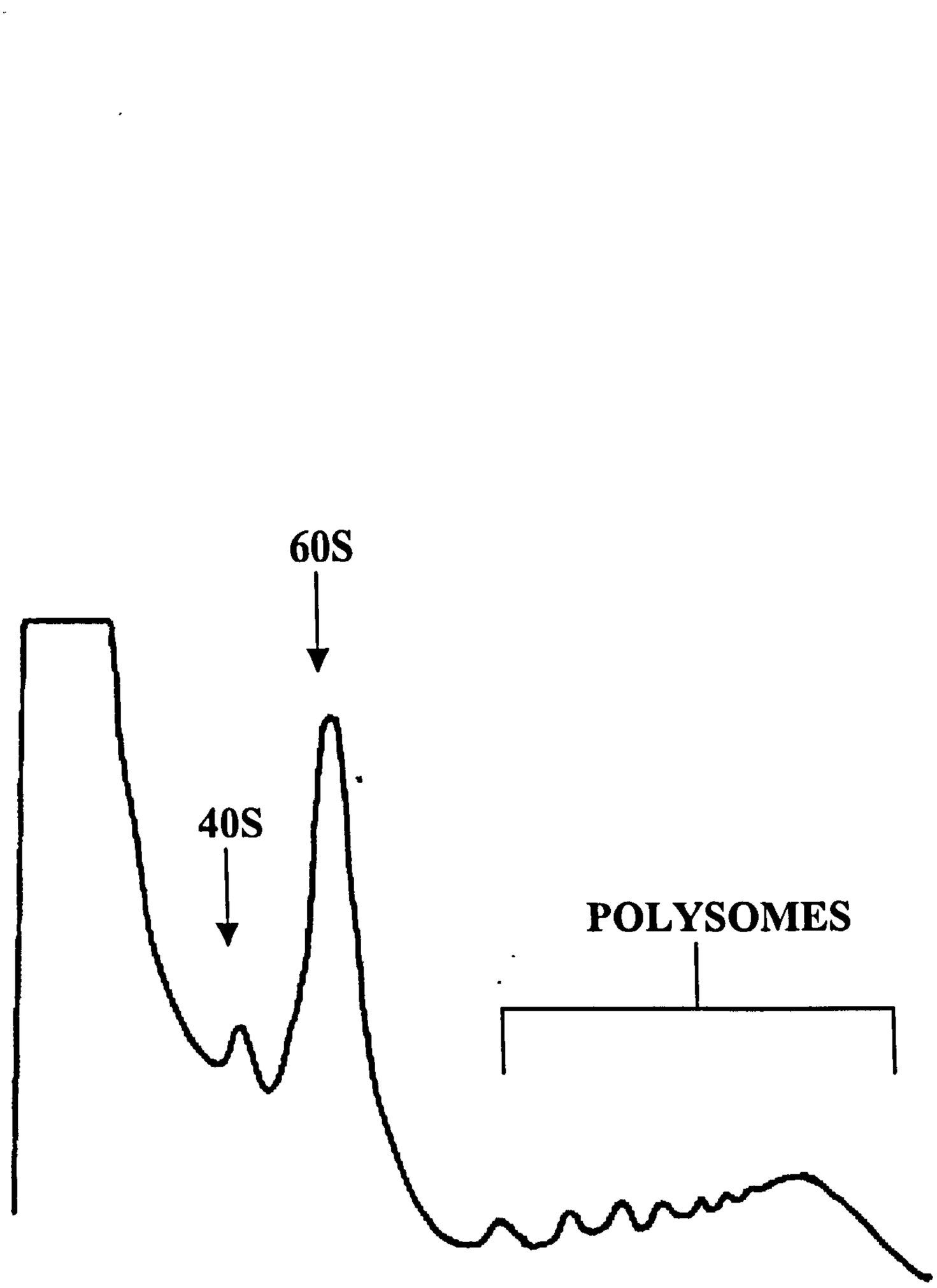


Figure 3.7 Polysome-subpolysome distribution in GM1953 cells
A postnuclear supernatant was prepared from 2.5 x10^7 GM1953 cells. After centrifugation through a 10-50% sucrose density gradient the A_{254} was monitored continuously. Sedimentation is from left to right

the case here. The polysome associated messages sediment lowest in the gradient. From left to right, each peak represents messages with an increasing number of ribosomes attached, starting with disomes (two ribosomes attached). GM1953 cells are immortalised and able to grow in culture continuously, therefore are expected to be actively translating.

Polysome profiles were recorded for tonsil CD19+ B cells and B-CLL cells (Figure 3.8). There were less polysome associated messages for these cells than the GM1953 cells, even though 10 fold more cells have been used. This was expected as primary cells tend to be more quiescent than immortalised cells. The profile for B-CLL cells was relatively similar to that for the tonsil CD19+ B cells, however there appeared to be slightly less polysome associated messages. This may reflect the quiescent phenotype observed for B-CLL cells (Andreeff et al., 1980; Caligaris-Cappio and Hamblin, 1999). However, the polysome-subpolysome distribution is not a direct measure of translation rates, therefore these observations are just speculative. The polysome profile does show that a pool of messages was being translated, so further analysis of this was required.

3.5 Northern analysis of B-CLL translation

Northern analysis was used to study the distribution of individual messages across the polysome profile. After centrifugation and recording of polysome profiles, sucrose density gradients were collected into 1ml fractions. RNA was isolated from fractions, using guanidine-hydrochloride and ethanol to precipitate RNA. This was then separated by denaturing gel electrophoresis and transferred to nitrocellulose. The distribution of actin, polyA binding protein (PABP) and ribosomal protein S16 was analysed for B-CLL cells, tonsil CD19+ B cells and GM1953 cells. The majority of actin mRNA was found in the polysome fractions for all three cell types (Figure 3.9). The presence of this message in the polysome fractions for all three cell types suggested that ribosomes had been successfully frozen onto mRNA molecules by the cycloheximde treatment.

Poly(A) binding protein (PABP) and ribosomal protein S16 (RPS16) were mostly found in the subpolysome factions for B-CLL cells and tonsil CD19+ B cells but were more polysomally associated in GM1953 cells (Figure 3.10). PABP and RPS16 are both 5'TOP messages, a subset of mRNAs that encode ribosomal proteins and other components of the translational machinery. Their expression is regulated at the translational level and they are highly expressed during active cell growth but their translation is turned off in quiescent

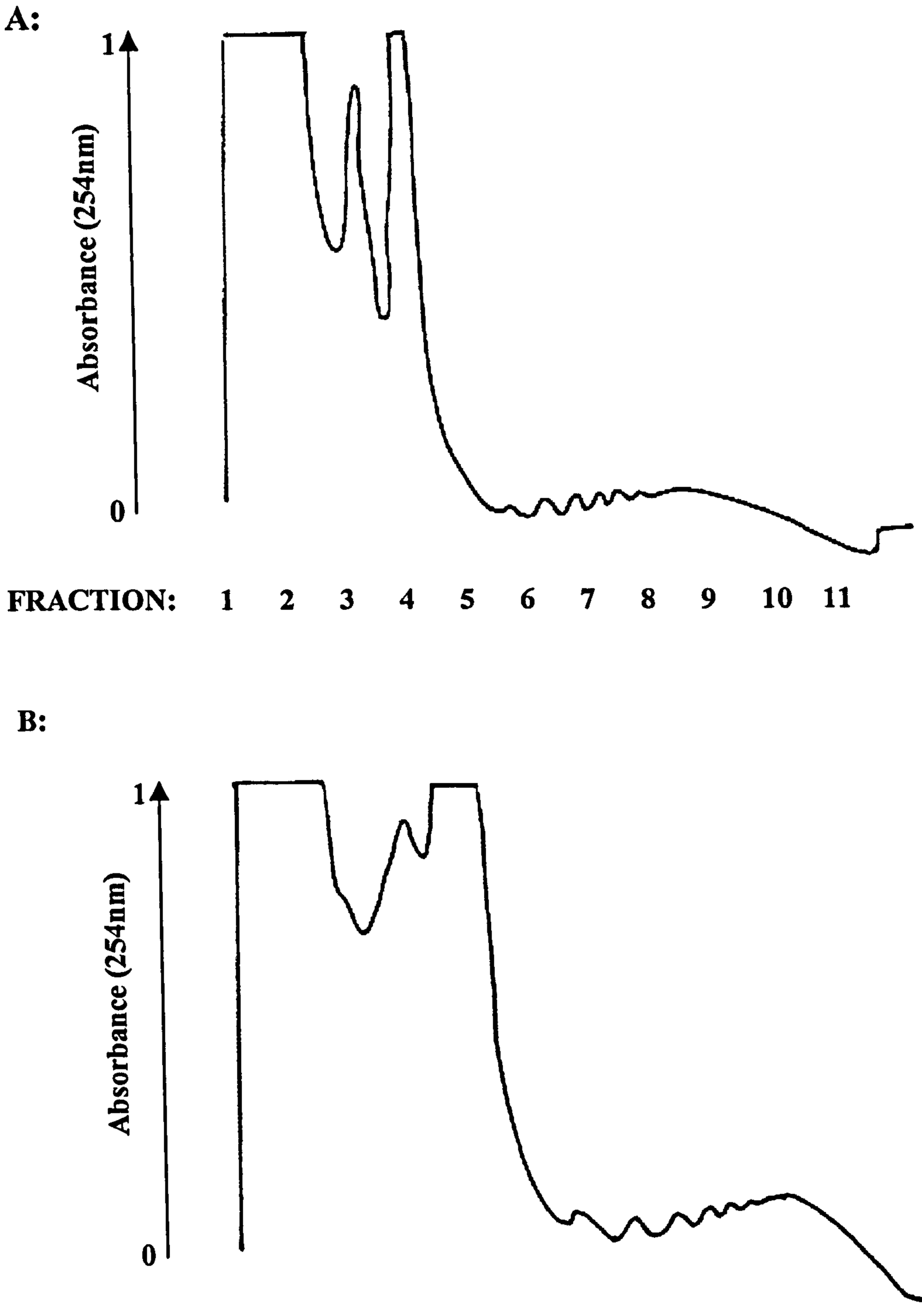


Figure 3.8 Polysome-subpolysome distribution in B-CLL (A) and tonsil CD19+ B cells (B). A postnuclear supernatant was prepared from about 3 x10^8 B-CLL (patient 1, representative of all patients) or tonsil CD19+ B cells. After centrifugation through a 10-50% sucrose density gradient the A_{254} was monitored continuously. Sedimentation is from left to right. A reduction in the amount of polysome associated mRNAs was observed for both cell types compared to GM1953 cells.

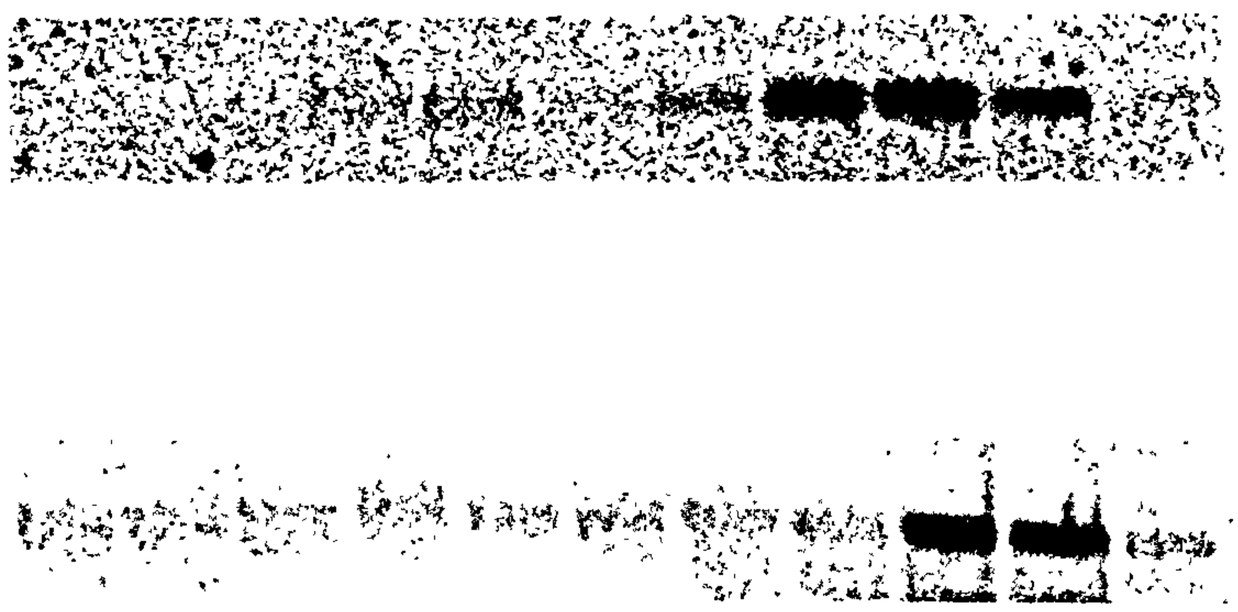
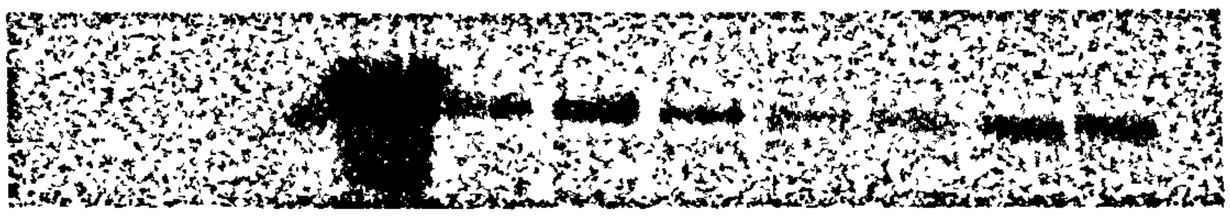
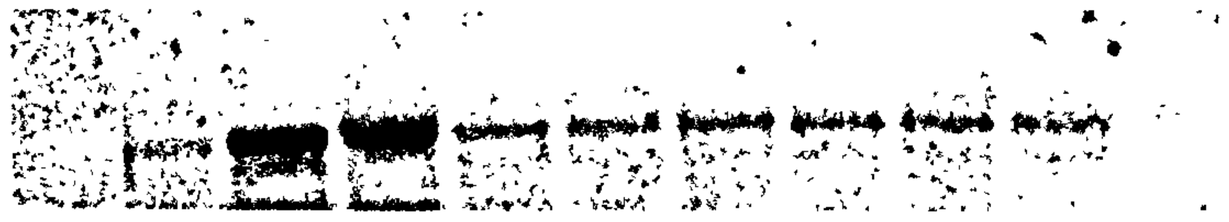


Figure 3.9 Northern analysis of B-CLL translation. RNA isolated from sucrose density gradient fractions for B-CLL cells (patient 1, representative of all patients), tonsil CD19+ B cells and GM1953 cells was used for Northern analysis. The distribution of mRNAs for actin was analysed and observed to be present in polysome fractions for all three cell types.

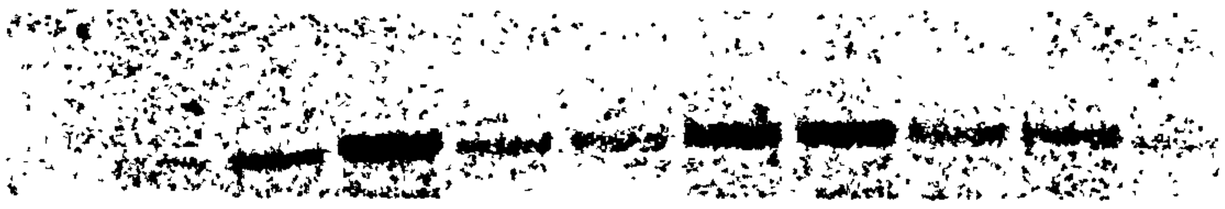
A: PABP



B-CLL cells



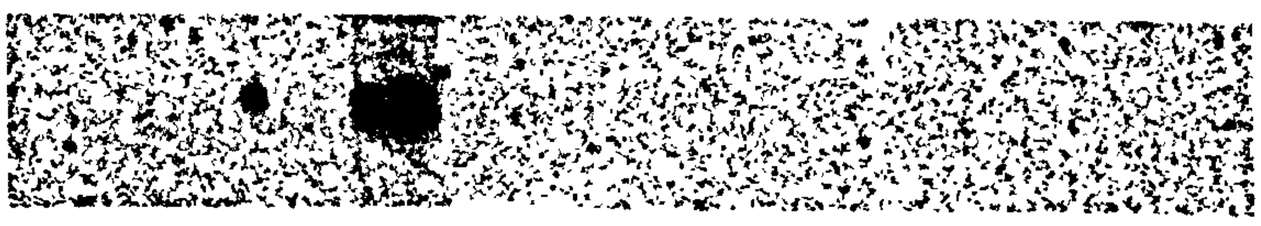
Tonsil CD19+ B cells



GM1953 cells

B: RPS16

B-CLL cells



Tonsil CD19+ B cells

GM1953 cells

Figure 3.10 Northern analysis of B-CLL translation. RNA isolated from sucrose density gradient fractions for B-CLL cells (patient 1, representative of all patients), tonsil CD19+ B cells and GM1953 cells was used for Northern analysis. The distribution of mRNAs for PABP (A) and RPS16 (B) was analysed. Both messages were polysome associated in GM1953 cells but subpolysomal in B-CLL and tonsil CD19+ B cells.

cells resulting in a shift of message from polysomes to subpolysomes (this is further discussed in section 1.4.4). The presence of PABP and RPS16 in the subpolysome fractions for B-CLL cells and CD19+ B cells suggested that they were quiescent, whereas for GM1953 cells both messages were more polysomally associated as these cells were actively growing.

3.6 An alternative polysome-subpolysome distribution for B-CLL cells

For a subset of B-CLL patients, a non-typical polysome-subpolysome distribution was obtained (figure 3.11). No peaks were observed for polysomal mRNA and the peaks for the subpolysomes were larger, implying that no translation was occurring in these cells. However, this could not have been the case for the cells to have been viable *in vivo*.

EDTA was added to the lysis buffer to determine whether the profile recorded represented the distribution of mRNAs between polysome and subpolysome pools in the cell. EDTA causes the release of ribosomes from mRNA molecules, therefore there should be a shift of material to the top of the gradient. Profiles of this type were recorded for B-CLL cells representing the two subgroups observed (figure 3.12). A shift of the peaks to the left was observed for both subgroups, therefore the profile recorded for this second subgroup was representative of the mRNA distribution. Further analysis was required to determine the nature of these cells and what was causing this unusual profile.

3.7 Analysis of RNA for patients with the alternative polysome-subpolysome distribution

The RNA was analysed from this subgroup of patients and compared to that for patients with a typical polysome-subpolysome distribution. RNA was isolated from gradient fractions and separated by denaturing agarose gel electrophoresis (Figure 3.13). Typically two bands should be observed on the gel, representing the 28S and 18S ribosomal RNAs (rRNAs), as seen for patients with a typical polysome-subpolysome distribution (Figure 3.13A). However, this was not observed for patients with the alternative distribution (Figure 3.13B). The rRNA appeared to have been cleaved at multiple sites and explained the absence of polysomes for these patient samples.

Further analysis was required to determine whether this cleavage was occurring before or after blood was taken. Total RNA was isolated from the lysate used for the polysome

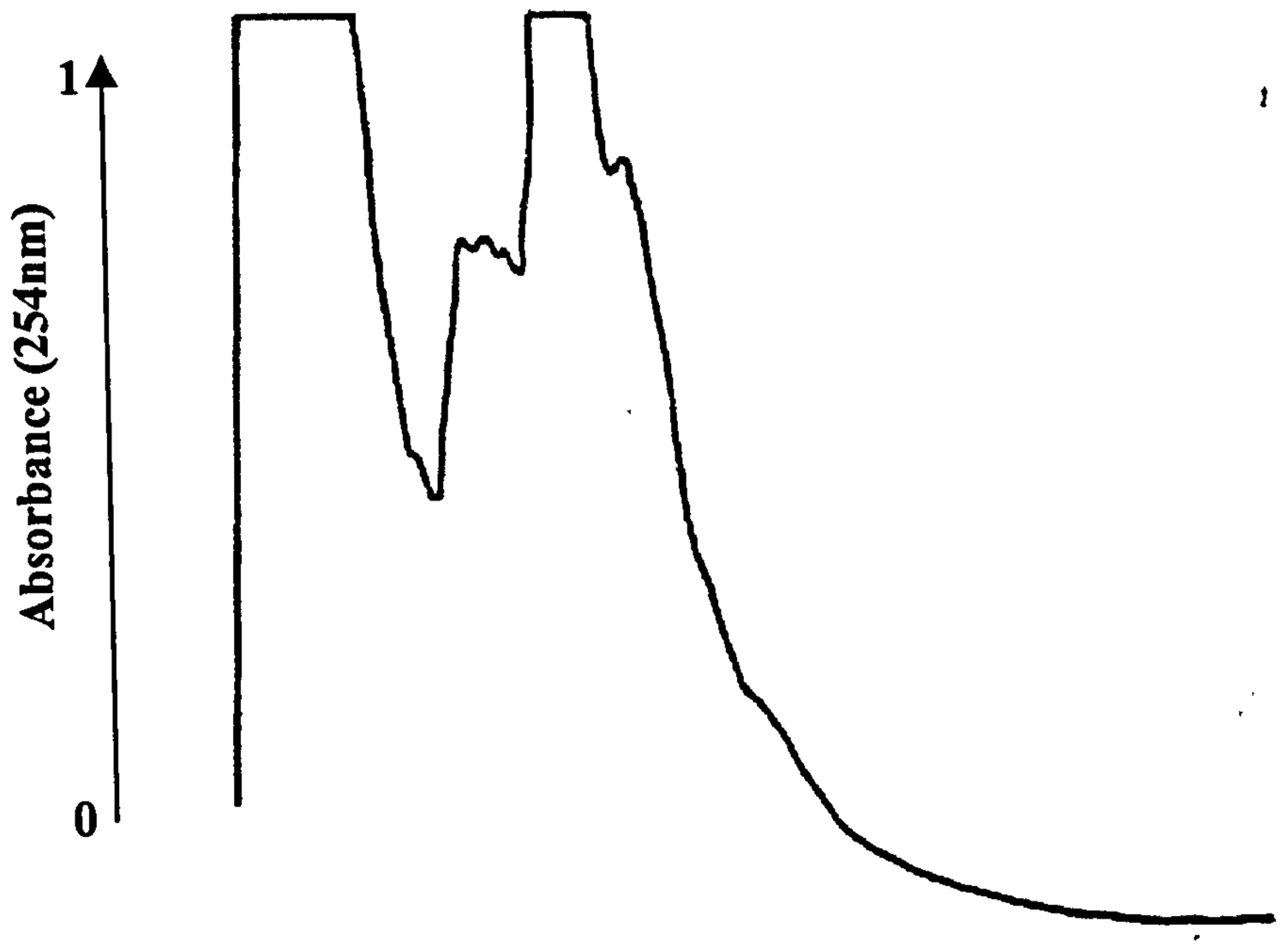


Figure 3.11 Alternative B-CLL polysome-subpolysome distribution. A postnuclear supernatant was prepared from B-CLL cells (patient 12). After centrifugation through a 10-50% sucrose density gradient the A_{254} was monitored continuously. Sedimentation is from left to right.

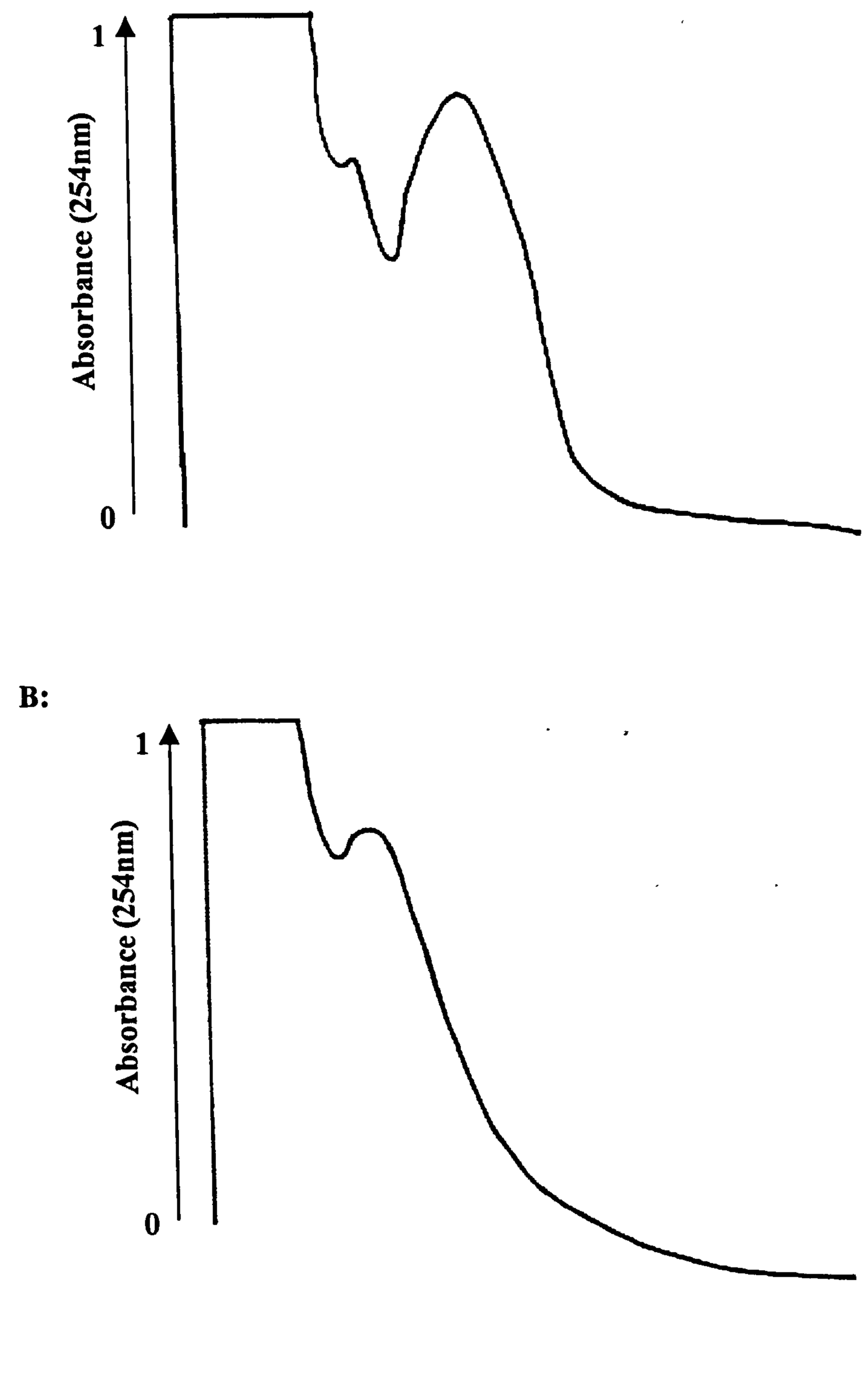


Figure 3.12 Polysome-subpolysome distribution after EDTA release of ribosomes. EDTA was added to the postnuclear supernatant for B-CLL patients of each subgroup before sucrose density gradient centrifugation. A_{254} was then monitored for each gradient. A shift of material to the top of the gradient was observed for both patients with a typical polysome-subpolysome distribution (A-patient 1) and non-typical (B-patient 12). Traces are representative of all patients.

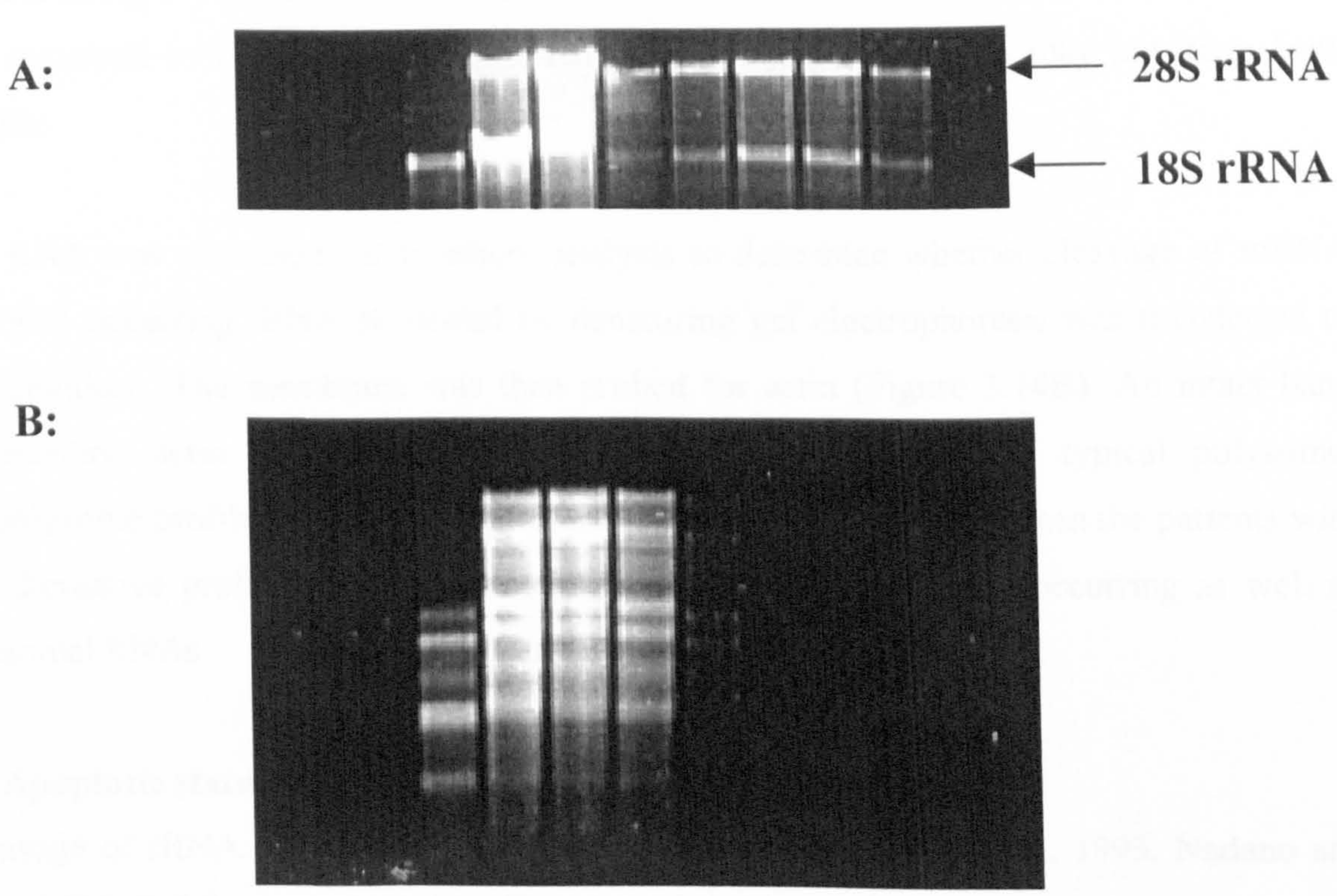


Figure 3.13 Denaturing gel electrophoresis of gradient RNA. RNA was isolated from sucrose density gradient fractions and subject to denaturing gel electrophoresis, for B-CLL patients with typical (A-patient 1) and non-typical (B-patient 12) polysome-subpolysome profiles. The ribosomal RNA for patients with non-typical polysome-subpolysome distributions was observed to have been cleaved. Representative of all patients.

analysis, and also directly from blood samples immediately after being taken, for both types of B-CLL patient, then separated by denaturing gel electrophoresis (Figure 3.14A). For patients with normal polysome-subpolysome profiles (patients 7, 8 and 10) the rRNA was intact for RNA isolated from both blood and lysate. For the patients with an alternative polysome-subpolysome profile (patients 12 and 13), rRNA from lysate samples was cleaved but was intact for that isolated from blood. Therefore it appears that the cleavage was occurring *in vitro* after samples were taken and RNA was intact *in vivo*. The cleavage also appeared to be different for the two patient samples used so may not have been specific.

The RNA was also used for Northern analysis to determine whether cleavage of mRNA was also occurring. RNA separated by denaturing gel electrophoresis was transferred to nitrocellulose. The membrane was then probed for actin (Figure 3.14B). An intact band representing actin mRNA was observed for the patients with a typical polysome-subpolysome profile. However no band was seen for lysate samples from the patients with the alternative profile, suggesting that the cleavage of mRNA was occurring as well as ribosomal RNAs.

3.8 Apoptotic status for patient samples with cleaved RNA

Cleavage of rRNA and mRNA occurs during apoptosis (Houge et al., 1993; Nadano and Sato, 2000; Del Prete et al., 2002). Therefore the cleavage observed in this subgroup of B-CLL patients could be as a result of apoptosis occurring during the purification procedure. Western analysis was performed on samples from both types of B-CLL patient to determine poly(ADP-ribose) polymerase (PARP) cleavage. PARP (116kDa) is cleaved during apoptosis into a 24kDa and 89kDa fragment. For both types of B-CLL patient only full length PARP was observed (Figure 3.15). This suggests that no apoptosis had occurred in either type of B-CLL sample during the purification process and was not the cause of the RNA cleavage observed.

3.9 Analysis of ZAP70 protein levels between the two subgroups

Progression of B-CLL is variable; some patients survive many years whilst others have a more aggressive form and die within a couple of years. This is thought to be linked to the mutational status of immunoglobulin heavy-chain variable (I_gV_H) genes (Damle et al., 1999; Hamblin et al., 1999). Microarray analysis revealed that expression of ZAP70

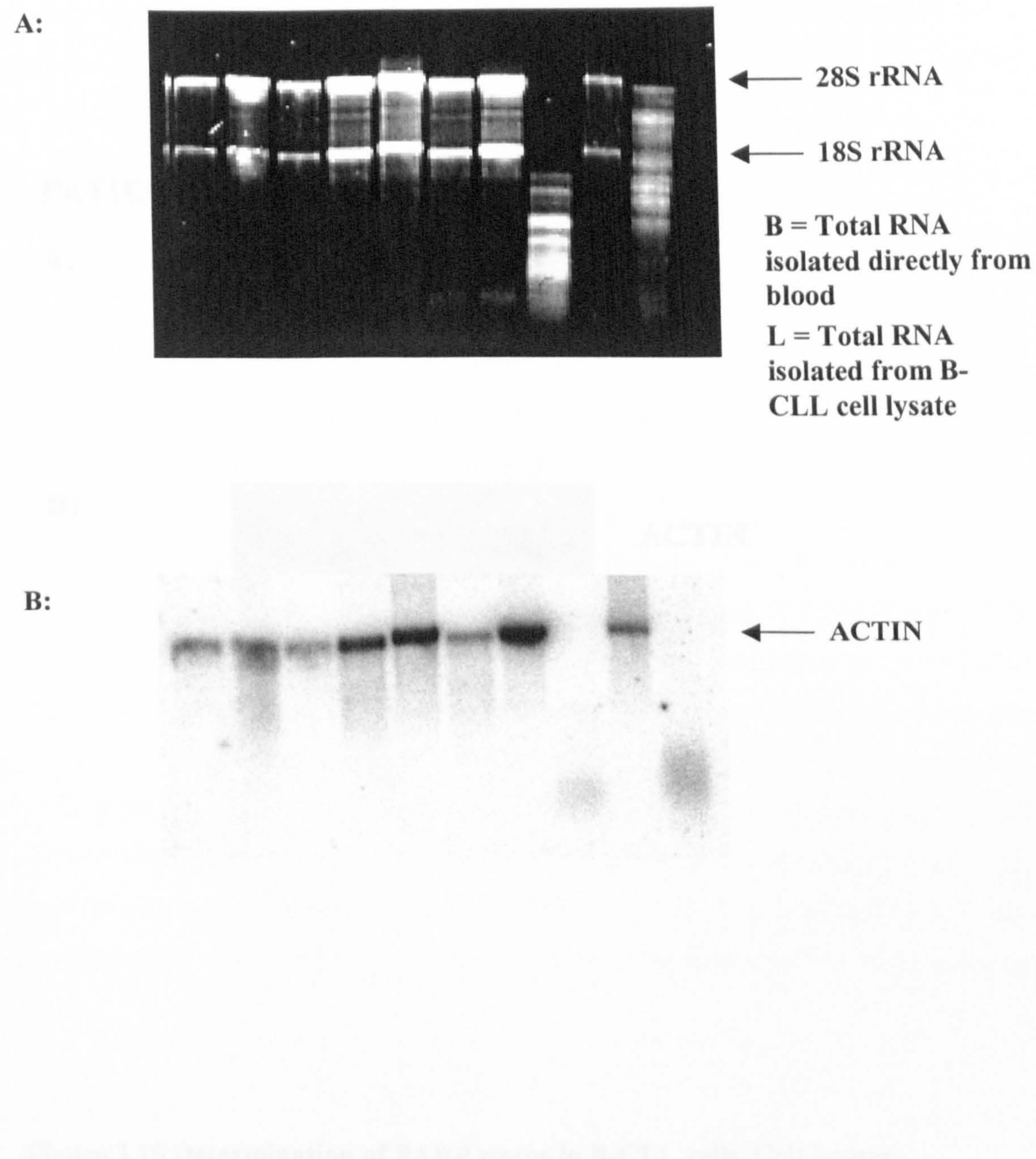


Figure 3.14 Analysis of Total RNA. Total RNA was isolated for B-CLL patients with typical (patients 8, 7 and 10) and non-typical (patients 12 and 13) polysome-subpolysome profiles, directly from blood and from B-CLL cell lysate, and subject to denaturing gel electrophoresis (A) and Northern analysis of actin mRNA levels (B). Only the RNA isolated from lysate was observed to have been cleaved. Plus the absence of a band for actin on the northern blot suggests mRNA was also cleaved.

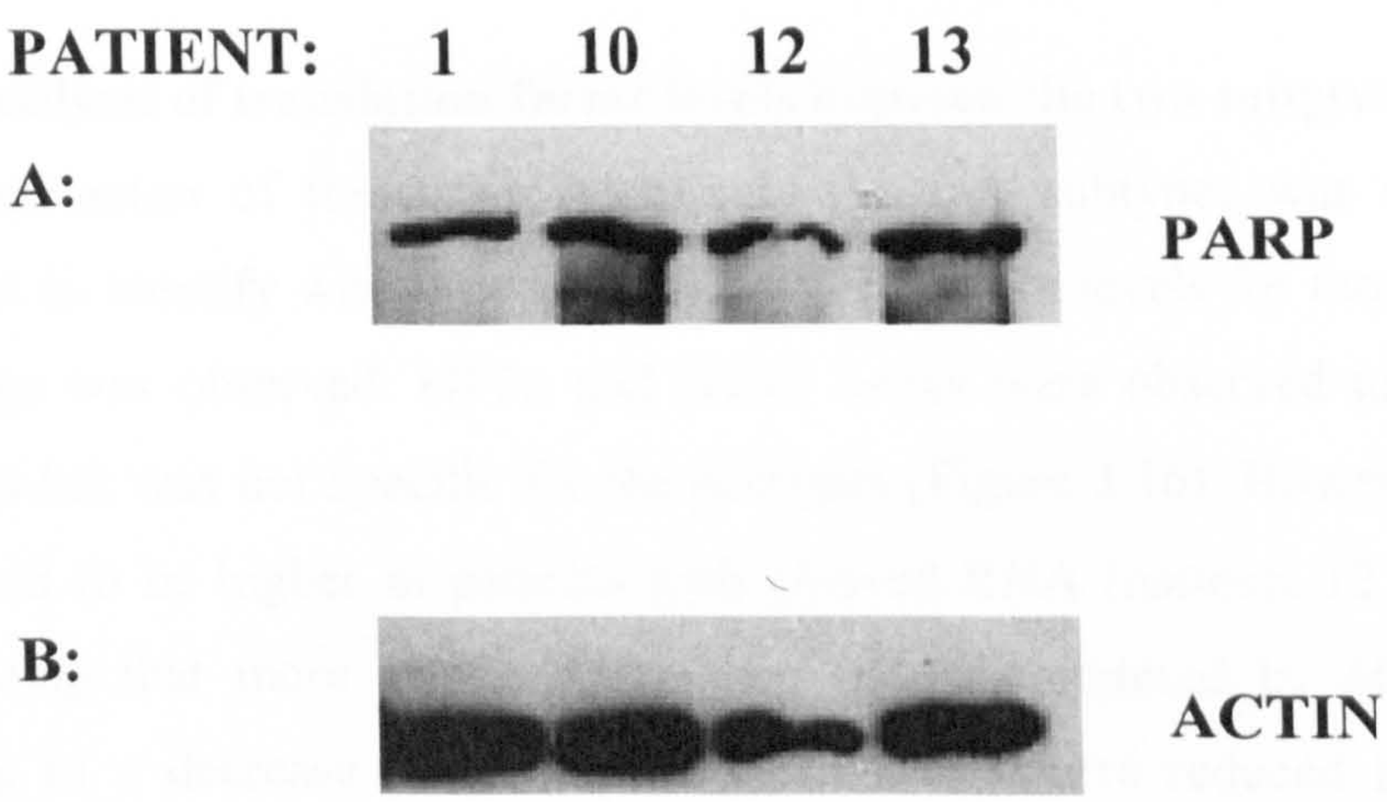


Figure 3.15 Determination of PARP status in B-CLL cells. Cell lysates were produced for B-CLL patients with intact (patients 1 and 10) and cleaved RNA (patients 12 and 13) and separated by SDS Page electrophoresis. These were then immunoblotted and probed with specific antibodies to poly(ADP-ribose) polymerase (PARP) (A) and actin (B). No cleavage of PARP was observed suggesting the cells were not apoptosing. Each Western was performed once.

correlated well with the I_gV_H mutational status (Klein et al., 2001; Rosenwald et al., 2001). Protein levels of ZAP70 also correlate with the mutational status and could potentially be used as a prognostic marker (Crespo et al., 2003). Western analysis was performed to determine whether the cleavage of RNA observed for a subgroup of B-CLL patients correlates with ZAP70 expression and therefore B-CLL disease progression (Figure 3.16). Variation of ZAP70 levels between the B-CLL patients was observed but this did not correlate with the cleavage of RNA.

3.10 Analysis of translation factor levels between the two subgroups

The expression of translation factors in the two subtypes was compared by Western analysis to identify whether there were differences in levels for those patients where RNA cleavage was observed. eIF2α and eIF4E levels were observed to vary between B-CLL patients but was not specific for the subtypes (Figure 3.16). However, 4EBP1 levels were observed to be higher in patients with cleaved RNA (patients 12 and 13) (Figure 3.17), suggesting that more eIF4E could have been sequestered by 4EBP1 in these patients leading to a decrease in translation. eEF2 levels were reduced in patients where RNA cleavage was observed which could lead to lower translation elongation rates (Figure 3.18). eEF2 kinase levels were only lower in one of the two patients and p70S6 kinase levels do not vary (Figure 3.18). As mentioned previously, a microarray study has identified two subgroups for B-CLL which differed in the level of expression for ribosome and translation associated genes at the level of transcription (Dürig et al., 2003), therefore differences observed here may correlate with this data. eEF2 was one factor identified in the study therefore the differences observed here could define the two subgroups and would also suggest that there was a correlation with the microarray data and the RNA cleavage observed.

3.11 Discussion

The translational status for B-CLL cells was examined to determine whether there were any general defects affecting global translation. The results obtained imply that there was a relatively low rate of protein synthesis occurring in B-CLL cells. Analysis of translation factor levels revealed reduced levels of eIF4E, an important factor for the initiation process. There was also variation in expression between patients, which could correlate with results from a microarray study that identified two subgroups of B-CLL differing in expression of ribosome and translation associated genes (Dürig et al., 2003). There were significantly less

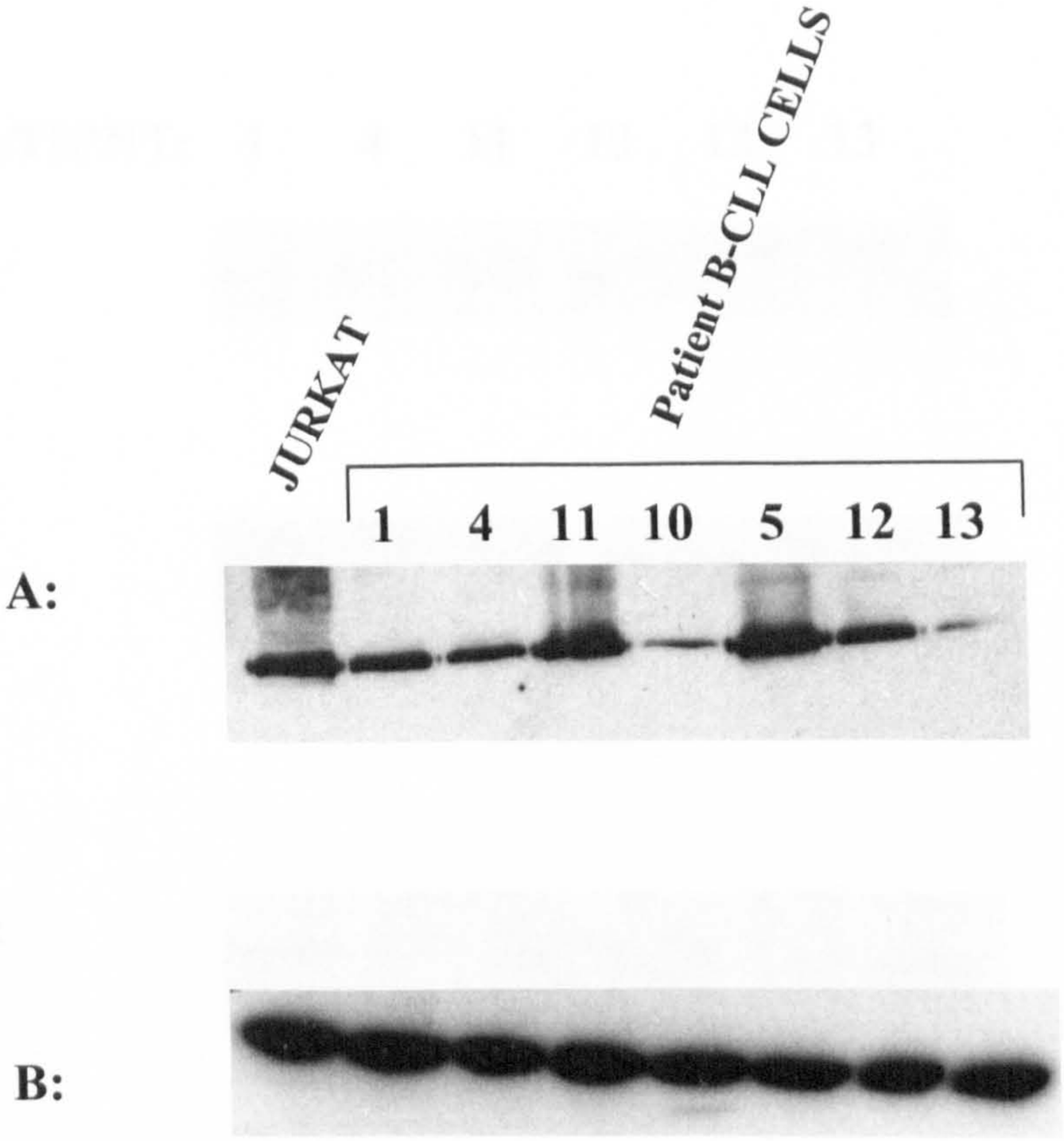


Figure 3.16 Determination of ZAP70 protein levels. Cell lysates were produced for B-CLL patients with intact (patients 1, 4, 11, 10 and 5) and cleaved RNA (patients 12 and 13) and separated by SDS Page electrophoresis. These were then immunoblotted and probed with specific antibodies to ZAP70 (A) and actin (B) (each Western was performed once). RNA cleavage was not observed to correlate with ZAP70 levels.

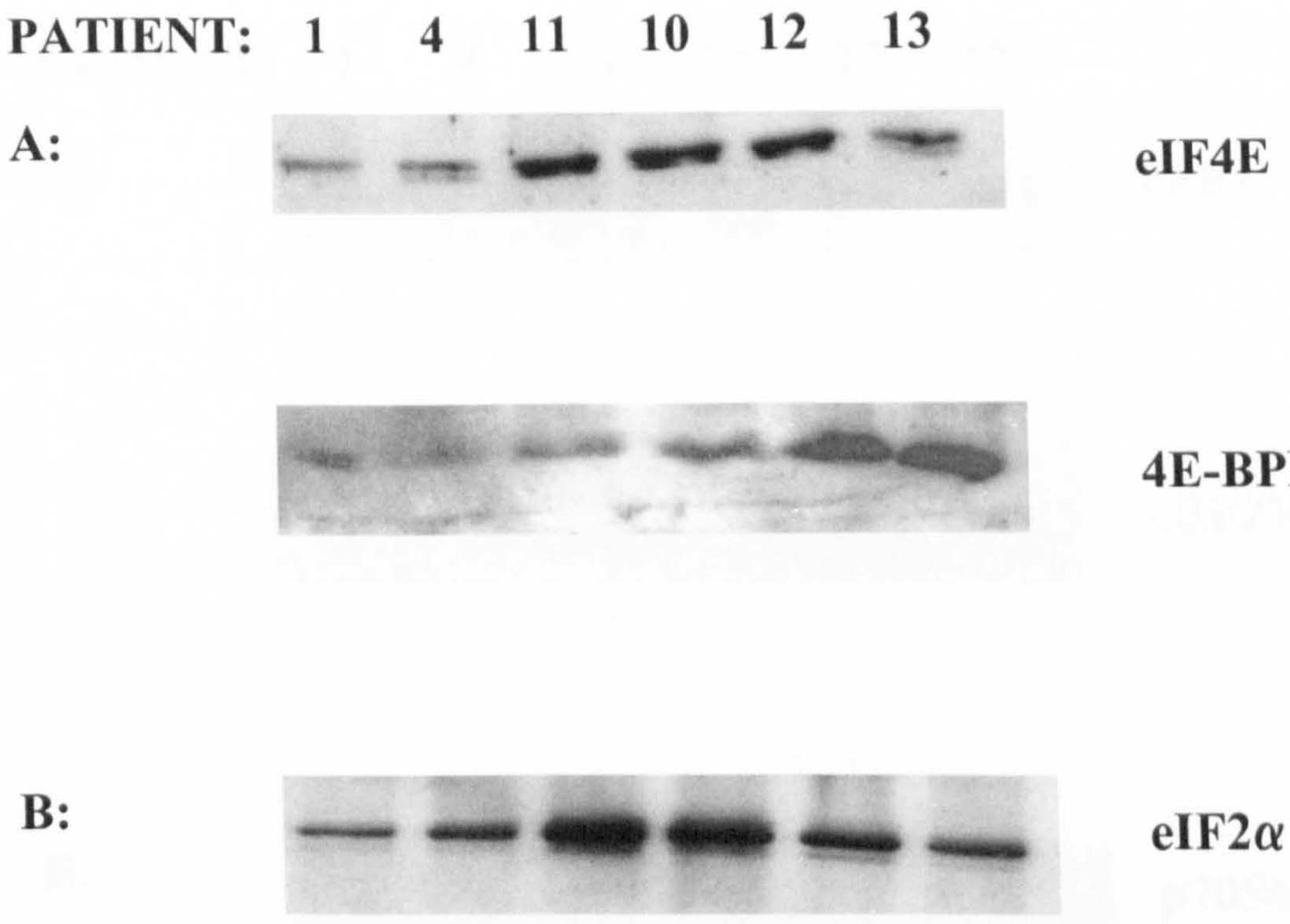


Figure 3.17 Determination of translation factors levels for subgroups. Cell lysates were produced for B-CLL patients with intact and cleaved RNA and separated by SDS Page electrophoresis. These were then immunoblotted and probed with specific antibodies to eIF4E, 4E-BPI (A), eIF2alpha (B) and actin. A higher protein level of 4E-BPI was observed for patients with cleaved RNA. Each Western was performed once.

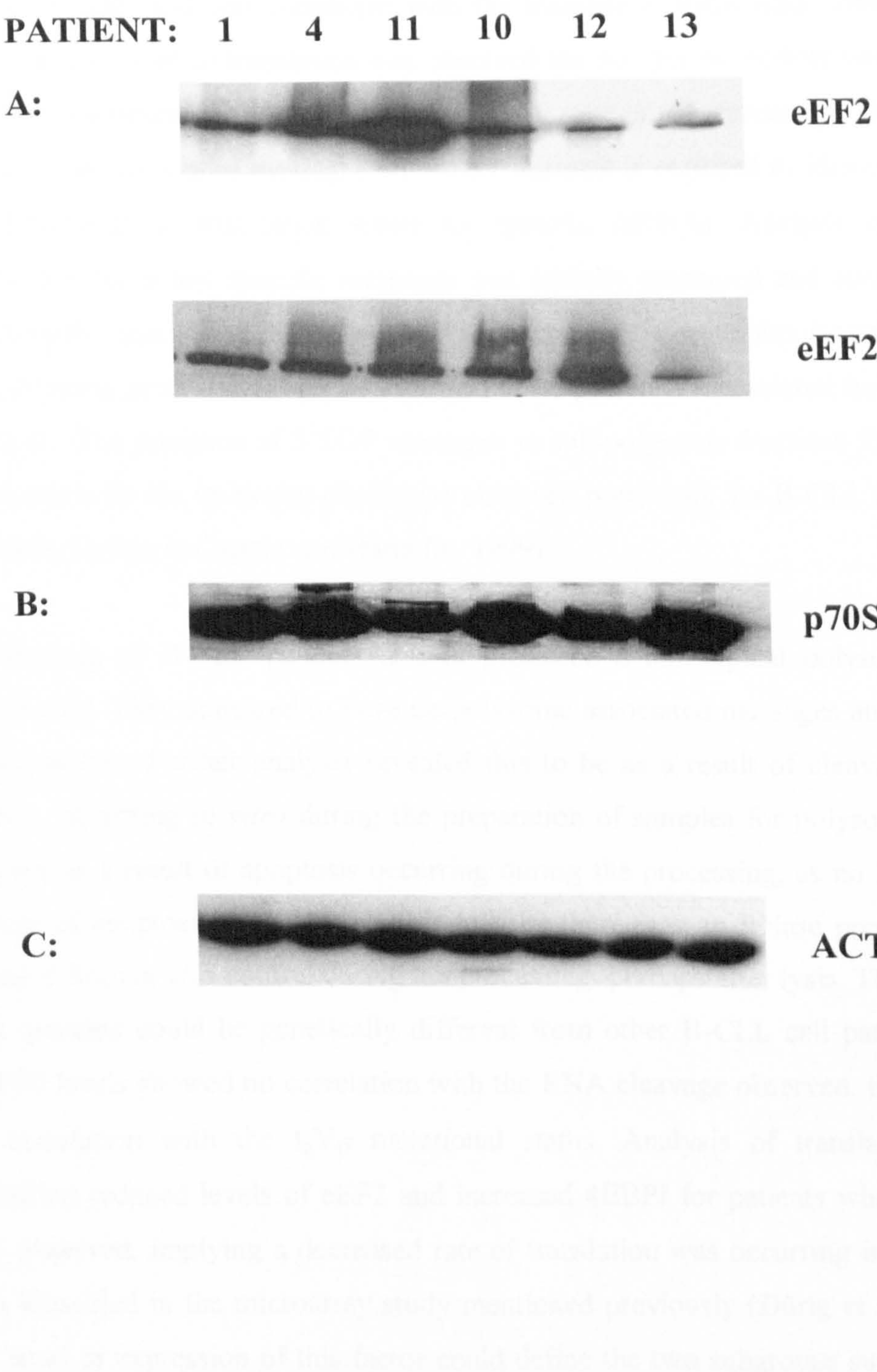


Figure 3.18 Determination of translation factors levels for subgroups. Cell lysates were produced for B-CLL patients with intact and cleaved RNA and separated by SDS Page electrophoresis. These were then immunoblotted and probed with specific antibodies to eEF2, eEF2K (A), p70S6K and actin (C). A Decreased protein levels of eEF2 was observed for patients with cleaved RNA. Each Western was performed once.

polysome associated messages for B-CLL cells than GM1953 and slightly less than for CD19+ B cells. This also suggests there was a reduced level of global translation occurring in B-CLL cells and was consistent with the translation factor data. Therefore overall, a relatively low level of translation was observed but no specific defects were identified that could be contributing to tumorigenesis. The polysome profile reveals the presence of a pool of polysome associated messages so further analysis is required to identify whether there are differences in translation levels for specific mRNAs. Analysis of the polysome association for a few specific messages was initially examined and revealed actin to be polysomally associated whereas 5'TOP messages were subpolysomal. Actin is a housekeeping gene and would be expected to be polysome associated for maintaining cell survival. The presence of 5'TOP messages in sub-polysome fractions for B-CLL cells is consistent with the quiescent phenotype observed previously for B-CLL cells (Andreeff et al., 1980; Caligaris-Cappio and Hamblin, 1999).

A subgroup of B-CLL patient samples produced a non-typical polysome-subpolysome distribution. They appeared to have no polysome associated messages and a larger pool of subpolysomes. Further analysis revealed this to be as a result of cleavage of rRNA and mRNA, occurring *in vitro* during the preparation of samples for polysome analysis. This was not as a result of apoptosis occurring during the processing, as no PARP cleavage, a marker of apoptosis, was observed. It may be there was an RNase present in these cells released from *in vivo* control during the processing, perhaps after lysis. This subgroup of B-CLL patients could be genetically different from other B-CLL cell patients. Analysis of ZAP70 levels showed no correlation with the RNA cleavage observed, therefore there was no correlation with the I_gV_H mutational status. Analysis of translation factor levels identified reduced levels of eEF2 and increased 4EBPI for patients where RNA cleavage was observed, implying a decreased rate of translation was occurring in these cells. eEF2 was identified in the microarray study mentioned previously (Dürig et al 2003), therefore the level of expression of this factor could define the two subgroups suggesting that RNA cleavage correlates with the expression of these ribosome and translation associated genes. Higher levels of mRNA for these factors was shown to correlate with a favourable clinical course therefore, as the RNA cleavage observed was indicated to correlate with reduced levels, this may represent patients with a more aggressive form of the disease.

Chapter 4

cDNA microarray analysis of B-CLL cell translation

4.1 Introduction

The data presented in chapter 3 suggests there is a reduced level of translation occurring in B-CLL cells. However, the polysome profiles revealed there was a pool of translated messages so there could have been changes in translation for specific mRNAs and further analysis was required. Microarray analysis is a powerful tool to use for screening expression of a large number of genes simultaneously and has previously been used to determine changes in B-CLL cells at the level of transcription (Aalto et al., 2001; Stratowa et al., 2001; Klein et al., 2001; Rosenwald et al., 2001; Jelinek et al., 2003). However it can also be used to screen for alterations in translation (Johannes et al., 1999). This chapter describes polysome profiling of B-CLL cells compared to control B cells.

4.2 Microarray experimental approach

To determine the translation status of mRNAs in cells using cDNA microarray, the mRNAs are separated into translated and untranslated pools. The translated pool is assumed to be mRNAs that are polysome associated and the untranslated messages subpolysome associated. This can be achieved using sucrose density gradient centrifugation, as described in section 3.4. Gradient fractions representing polysome and subpolysome associated mRNAs were pooled, as determined by analysis of polysome profiles and position of specific messages (Figure 4.1). Using this data, it was decided to pool fractions 1-6 for subpolysome mRNAs and 8-11 for polysome mRNAs. Fluorescently labelled cDNA was then synthesised for each pool, labelling one with cy3 and the other with cy5 and these jointly hybridised together on a microarray (Figure 4.1). Following hybridisation, the microarray slides were scanned and images captured electronically. Numerical values for fluorescence intensities were obtained using GenePix Pro 3.0 and were expressed as a ratio of cy5/cy3 for each gene on the array (7513 in total), which represents the ratio of subpolysome/polysome mRNA. Cy3 and cy5 fluorescence was measured at 532nm and 635nm respectively. Microarrays were performed in triplicate for 11 B-CLL patients, GM01953 cells and two pools of CD19+ B cells isolated from tonsil samples donated by healthy volunteers (three tonsils for each pool). At least one array of each three had reversed labelling of cDNA for subpolysomes and polysomes to counteract dye bias that

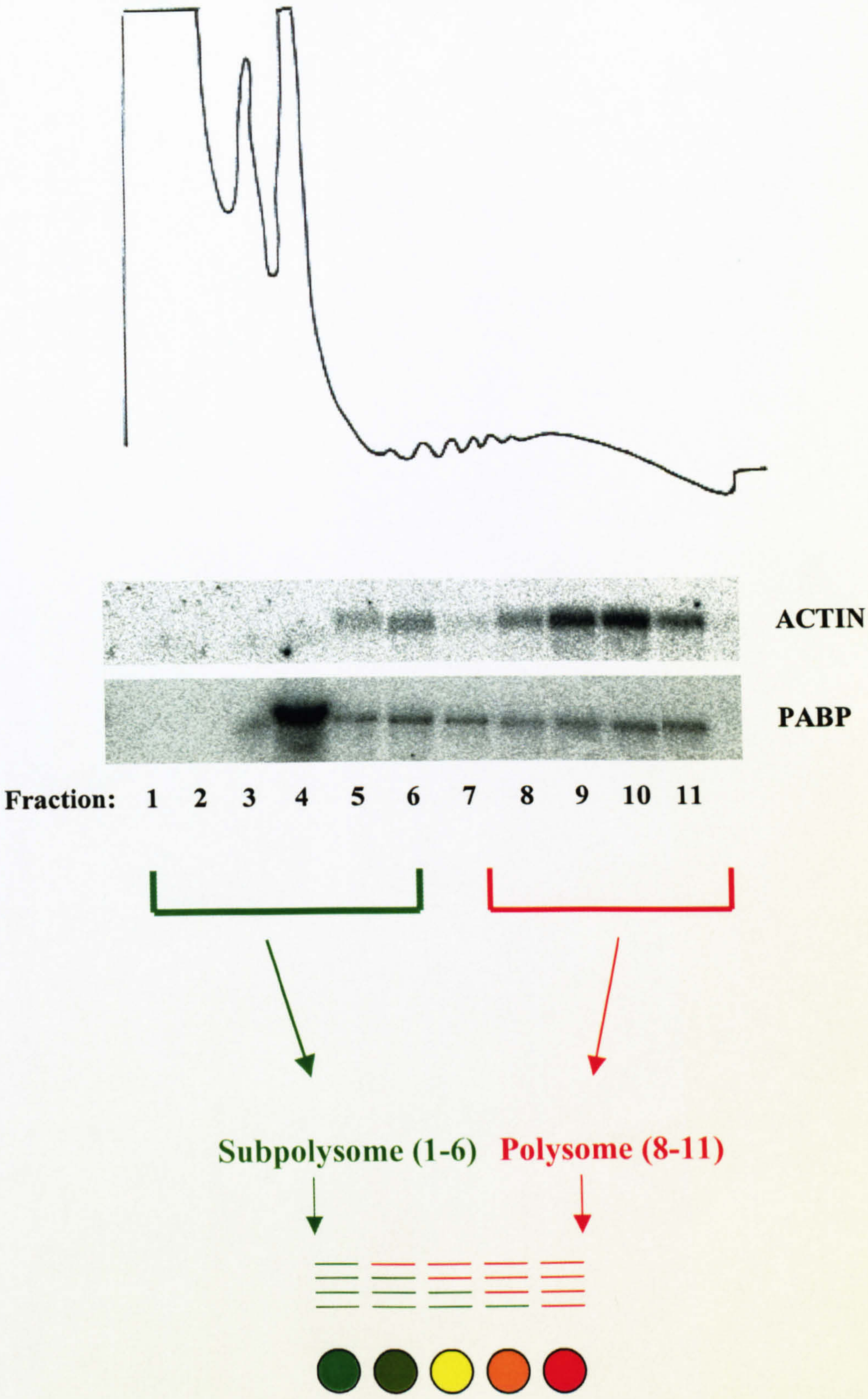


Figure 4.1 Microarray experimental design. Microarrays for B-CLL and tonsil CD19+ B cells have been performed using subpolysome and polysome RNA. RNA isolated from gradient fractions was used for the two pools as shown above. Fluorescently labelled cDNA was made for each pool, labelling subpolysomes green and polysomes red, and then hybridised to a microarray. Microarrays were performed for GM1953 cells, 11 B-CLL patients and two pools of tonsil CD19+ B cells (each a combination of samples from three healthy volunteers).

can occur. The first step for analysis was to obtain a ratio of subpolysome to polysome mRNA for each B-CLL patient sample, GM01953 cells and each tonsil pool. Data for each hybridisation was normalised, then a T-test performed for the three arrays in each triplicate. The data produced for B-CLL patient samples was then compared to that of the control. A second T-test was performed to compare the subpolysome/polysome ratios and thus identifying any differences in the amount of polysome associated message. Genes where the p value was less than 0.05 were selected as those with a significant change in the subpolysome/polysome ratio, and therefore amount of polysome associated message, for B-CLL patient samples.

The data produced for B-CLL cells was initially compared to data for GM01953 cells. 1982 genes were identified with significantly changed polysome association, 909 of which had increased polysome association and 1073 decreased polysome association (Appendices 1 and 2). This is a large number of genes found to be different so many of the differences probably reflect the immortalised phenotype of the GM1953 cells compared to the primary B-CLL cells, rather than a relevance to the disease. Therefore GM01953 cells do not make a suitable control for comparison to B-CLL. The B-CLL microarray data was then analysed against that for tonsil CD19+ B cells. 493 genes were identified with differential polysome association, 181 with increased association (Appendix 3) and 312 with decreased (Appendix 4), a more reasonable number of genes to be identified with a change in translation. Like B-CLL cells, the CD19+ B cells are primary so are a more suitable control to use, and the mRNAs identified as having a change in polysome association are more likely to be relevant to the biology of the disease. However, this is not the cell of origin for B-CLL so some changes identified may reflect this fact rather than being relevant to tumorigenesis. However there appears to be good correlation to the biology of B-CLL for the genes identified with significantly changed polysome association, which could therefore provide increased understanding of disease characteristics and lead to the development of new treatments.

4.3 Apoptotic resistance

Accumulation of B-CLL cells is thought to be a consequence of defective apoptosis. There are a number of genes identified that have anti-apoptotic activity and thus fit with this phenotype (Figure 4.2 and Appendix 3.3). For example Bcl2, which has previously been observed to be overexpressed in B-CLL cells at the level of transcription (Hanada et al.,

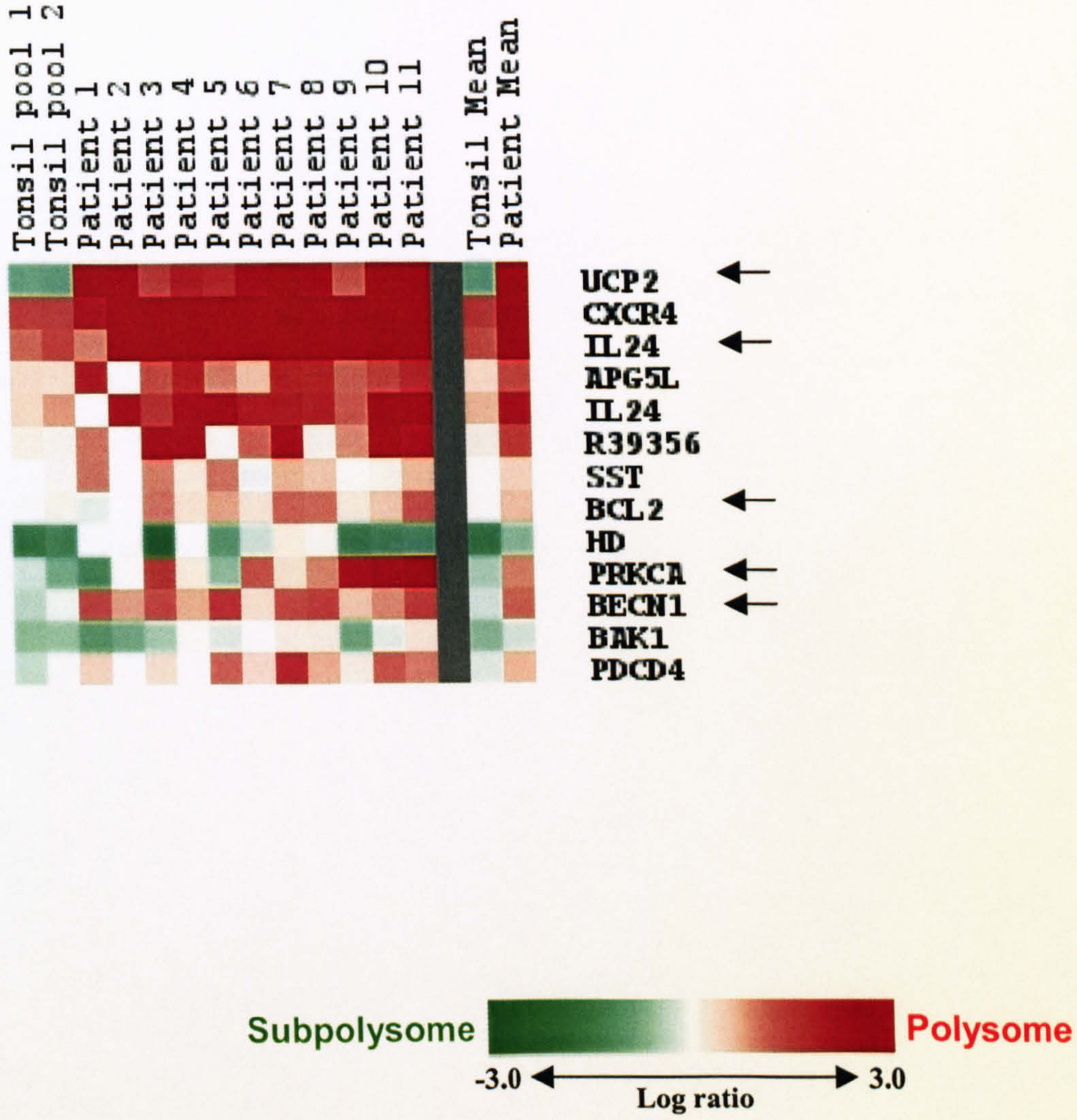


Figure 4.2 Apoptotic genes with increased polysome association in B-CLL cells, when compared to tonsil CD19+ B cells. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated. Genes which may have an anti-apoptotic role are highlighted by arrows.

1993; Robertson et al., 1996), was identified as having increased polysome association (Figure 4.2 and Appendix 3.3). An IRES element has recently been identified in the 5'UTR of Bcl2 mRNA (Sherrill et al., 2004), which could potentially be utilised as a method for upregulation of Bcl2 translation in B-CLL cells. In contrast there are a number of genes with pro-apoptotic activity, which have decreased polysome association for B-CLL (Figure 4.3 and Appendix 4.3). SIVA (CD27 binding protein) aids the apoptotic signalling of CD27, a receptor belonging to the TNF superfamily, through association with its cytoplasmic tail (Prasad et al., 1997; Xue et al., 2002). Overexpression of SIVA also induces apoptosis in a number of different cell lines (Prasad et al., 1997). PDCD2 and PDCD5 have been shown to have pro-apoptotic activity although their exact functions are unknown. PDCD5 has been reported to accelerate apoptosis in tumour cells (Liu et al., 1999), whilst the rat homolog of PDCD2 was observed to be associated with apoptosis in thymocytes (Owens et al., 1991).

There were also some other genes with increased polysome association where there role in apoptosis is not so well defined. UCP2 (uncoupling protein 2) is a member of a family of mitochondrial inner membrane carriers that are able to uncouple respiration from ATP synthesis by dissipating the electrochemical gradient. UCP2 is a negative regulator of reactive oxygen species production (Arsenijevic et al., 2000). It acts as a sensor of mitochondrial stress and is activated by superoxide or the subsequently formed lipid peroxidation products (Echtay et al., 2002; Echtay et al., 2003). There is evidence suggesting a cytoprotective role of UCP2 through limiting oxidative injury as overexpression of UCP2 in cardiomyocytes protected them from oxidative stress (Teshima et al., 2003). In addition, UCP2 can reduce cell death and caspase 3 activation induced by glucose and oxygen deprivation in neuronal cells (Mattiasson et al., 2003). Translational regulation may play a role in control of UCP2 gene expression. A short uORF has been identified in the 5'UTR of UCP2, which is inhibitory to translation (Pecqueur et al., 2001). UCP2 was observed to have increased polysome association in the mouse brain after focal ischemia (MacManus et al., 2004). This was under conditions where global translation was reduced, although the mechanism of upregulation was not deduced. Moreover, a recent report has observed that hnRNPK could bind to UCP2 mRNA at sites in the 3'UTR, and this enhanced insulin-mediated translation of the message (Ostrowski et al., 2004).

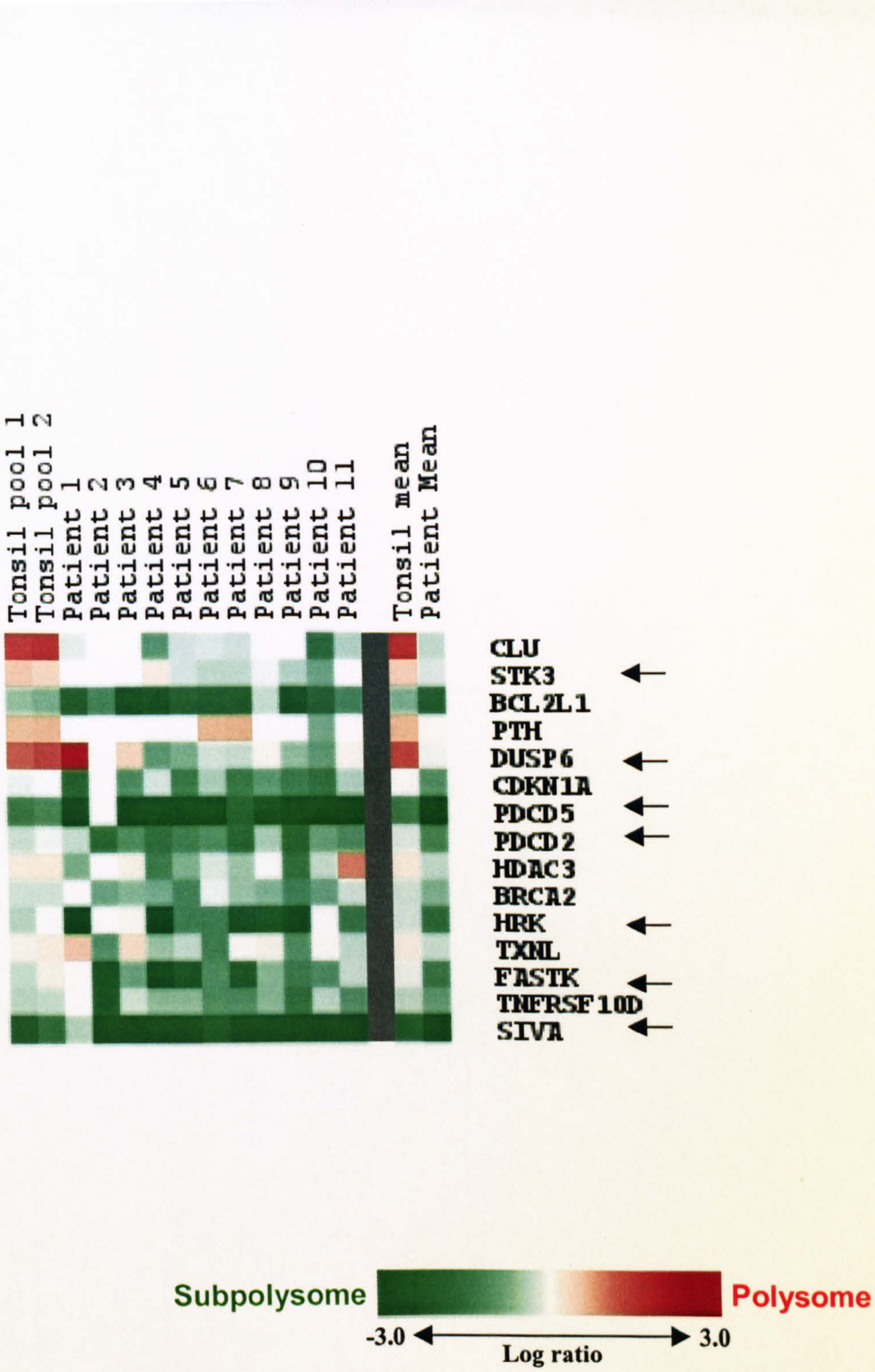


Figure 4.3 Apoptotic genes with decreased polysome association in B-CLL cells when compared to tonsil CD19+ B cells. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated. Genes which may have a pro-apoptotic role are highlighted by arrows.

IL24 (interleukin 24) was observed to have increased polysome association (Figure 4.3 and Appendix 3.3). It has also been identified as being up-regulated in a transcription microarray study for B-CLL (Jelinek et al., 2003). IL24 has been shown to suppress growth and induce apoptosis in a number of cancer cell types, including lung and prostate cancer (Huang et al., 2001; Jiang et al., 1996; Lebedeva et al., 2002; Saeki et al., 2000), however, overexpression in normal cells does not have this effect (Chada et al., 2004; Mhashikar et al., 2001; Lebedeva et al., 2003). The mechanism for growth suppression and apoptotic induction has not yet been elucidated, but IL24 could potentially cause the growth arrest observed in B-CLL cells. IL24 has recently been shown to also potentially have an anti-apoptotic role. It was found that IL24 could inhibit TNFα induced apoptosis by enhancing TNF mediated activation of NF-κB and NF-κB regulated expression of cyclin D1 and COX-2 (Aggarwal et al., 2004).

PDCD4 (programmed cell death 4) was identified as having increased expression during apoptosis induced by various stimuli in murine cells (Shibahara et al., 1995). Its exact function during apoptosis has not been defined, however it was recently observed that transient overexpression in T-47 and MCF-7 cells can induce apoptosis (Afonja et al., 2004). PDCD4 has also been shown to have a tumour suppressor function in a mouse keratinocyte model of tumour promotion and in the development of lung cancer (Cmarik et al., 1999; Chen et al., 2003). Interestingly, PDCD4 may partially exert its tumour suppressor function through an inhibition of translation. PDCD4 has been observed to bind to eIF4A and inhibit its helicase activity, and bind to eIF4G (Goke et al., 2002; Yang et al., 2003). This resulted in an inhibition of cap-dependent translation, however it appeared that IRES mediated translation was unaffected. Carbonic anhydrase II has been identified as a specific target for translational repression by PDCD4 and this contributes to its inhibition of endocrine tumour cell growth (Lankat-Buttgereit, et al 2004). PDCD4 could cause growth suppression of B-CLL cells through inhibition of translation, which is consistent with the reduced translation phenotype observed.

4.4 Cell cycle status

The observation that B-CLL cells are found to be quiescent (Andreeff et al., 1980; Caligaris-Cappio and Hamblin, 1999) could be due to cell cycle defects that block progression into the cell cycle. A number of the genes identified from the microarray analysis may fufill this role. BTG1 (B cell translocation gene 1), an anti-proliferative

protein, was found to have increased polysome association in B-CLL cells (Figure 4.4 and Appendix 3.5). BTG1 was discovered as part of a translocation in a case of B-CLL (Rimokh et al., 1991), however this is probably not a common mechanism of upregulation for BTG1 utilised in B-CLL as translocations are rare for this disease. The expression of BTG1 is growth regulated; it is highly expressed during the G0/G1 phases of the cell cycle then downregulated as G1 progresses (Rouault et al., 1992), and it can have anti-proliferative activity on cells. BTG1 is expressed in most tissues and cancer cells but has been shown to have elevated expression in B-CLL cells and other cells of lymphoid origin (Rouault et al., 1992). The mechanism of proliferation inhibition has not been deduced although it may involve interaction with other proteins such as PRMT1, an arginine N-methyltransferase, which has been observed to enhance the activity of BTG1 (Lin et al., 1996).

The microarray analysis revealed cyclin D3 to have decreased polysome association in B-CLL cells (Figure 4.5 and Appendix 4.4). D type cyclins are involved in the G1/S transition of the cell cycle therefore alterations in the levels of these proteins may be an important determinant of the cell cycle status of B-CLL cells. Mammals have three D type cyclins, D1, D2 and D3. Not all cells express all three types and there is evidence for specific roles for each, which are dependent on abundance and cell type. Cyclin D3 has been observed to be down-regulated in lymphoid cells in response to cAMP, which causes growth inhibition. This occurs at the level of translation elongation and may be due to phosphorylation of eEF2 (Naderi et al., 2000; Gutzkow et al., 2003). Further analysis of D-type cyclins is discussed in section 4.5.

WEE1 mRNA was observed to have increased polysome association in B-CLL cells (Figure 4.4 and Appendix 3.4). WEE1, in contrast to D type cyclins, is involved in the G2/M cell cycle transition. It plays an inhibitory role through its phosphorylation of CDC2 and prevents early entry into mitosis. Subsequent dephosphorylation of CDC2 is required for cell cycle progression to continue (Kellogg, 2003). Therefore, upregulation of WEE1 could cause cell cycle arrest at the G2/M transition as observed by Chang et al (2004). It is not clear how WEE1 would affect the cell cycle status of B-CLL cells as it is not involved in G1/S cell cycle transition. However, it has been reported that WEE1 is required for G2 delay, induced by partial inhibition of protein synthesis in yeast and WEE1 is up-regulated upon protein synthesis inhibition (Suda et al., 2000). Reduced protein synthesis in B-CLL

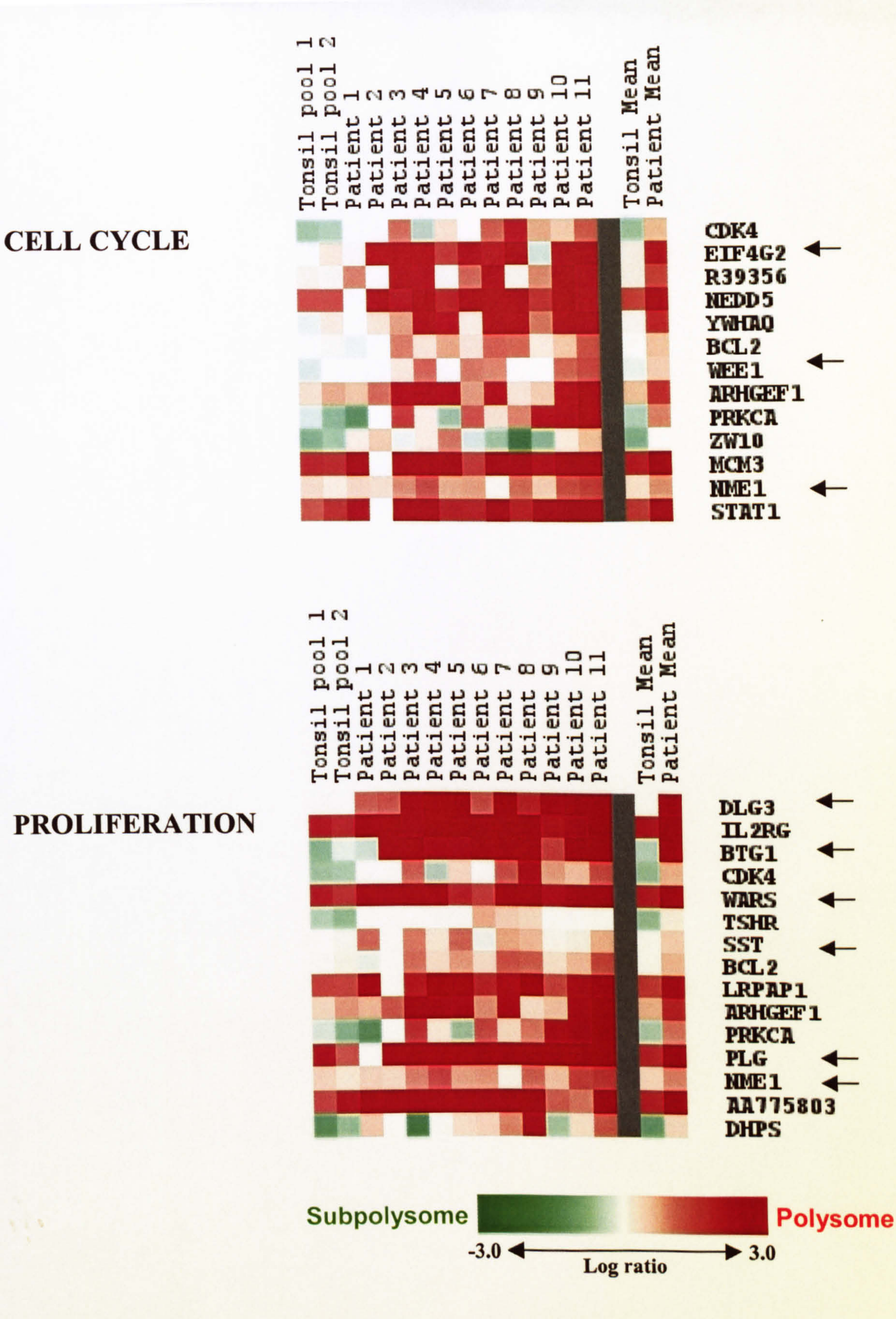


Figure 4.4 Cell cycle and proliferation genes with increased polysome association in B-CLL cells, when compared to tonsil CD19+ B cells. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated. Genes which may be involved with cell cycle arrest are highlighted by arrows.

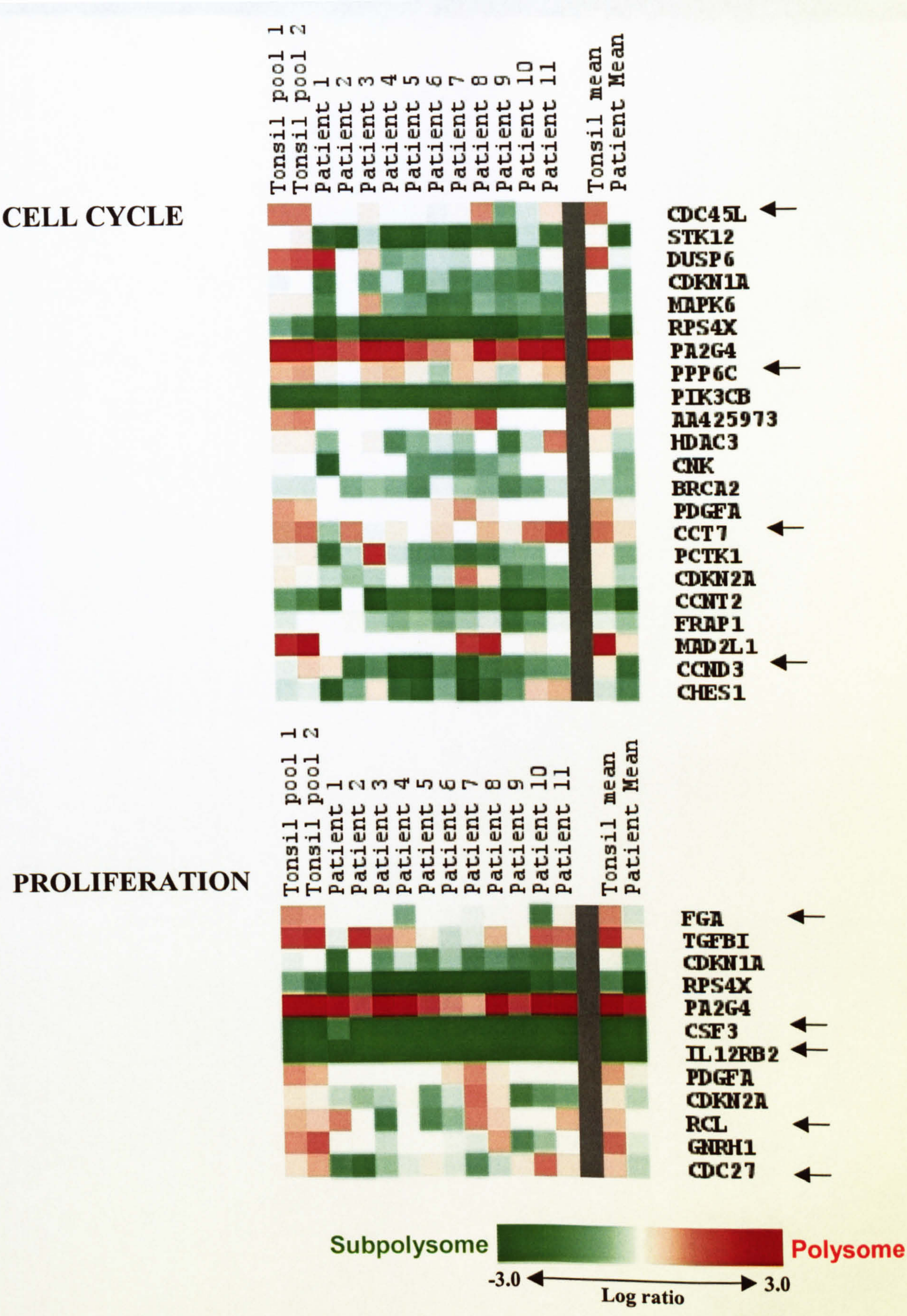


Figure 4.5 Cell cycle and proliferation genes with decreased polysome association in B-CLL cells, when compared to tonsil CD19+ B cells. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated. Genes which may be involved in cell cycle arrest are highlighted by arrows.

cells, as suggested by the data presented in chapter 3, could therefore cause up-regulation of WEE1.

4.5 B-CLL cell survival

B-CLL cells are observed to undergo apoptosis when cultured *in vitro* (Collins et al., 1989; MacFarlane et al., 2002), and are therefore thought to require signals from the *in vivo* microenvironment for their survival. Receptors involved in survival signalling may be upregulated in B-CLL cells. CXCR4 (chemokine receptor 4) had increased polysome association in B-CLL cells (Figure 4.6 and Appendix 3.6), plus has previously been shown to be overexpressed in B-CLL cells (Burger et al., 1999; Mohle et al., 1999; Durig et al., 2001). This receptor may have a role in survival of B-CLL cells as discussed in section 1.2.4. Likewise, there is evidence that interleukins may also play a role in B-CLL survival. IL2RG (interleukin 2 receptor gamma) had increased polysome association in B-CLL cells (Figure 4.6). It is also known as the common gamma chain (γc) as it is a functional component of a number of the interleukin receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. Several cytokines have been shown to play a role in survival of B-CLL cells *in vitro*, including some whose receptors include γc (Meinhardt et al., 1999). (Further discussed in section 1.2.4).

A number of integrins have a change in polysome association for B-CLL cells; ITGB7 had increased (Figure 4.6 and Appendix 3.6) and ITGA2, ITGA3 and ITGA7 decreased association (Figure 4.7 and Appendix 4.6). Integrins are heterodimeric glycoproteins, consisting of noncovalently associated α and ß subunits that play diverse roles in cell-cell and cell-matrix interactions, through binding to specific ligands. A number of studies have previously examined integrin expression in B-CLL. The ß1 integrin has been observed to be consistently expressed in B-CLL cells, associated with either α3, α4 or α5 (Vincent et al., 1996). The α4ß1 integrin has a role in B cell development. α4ß1 mediates interactions with fibronectin (FN) in the ECM and with vascular cell adhesion molecule (VCAM)-1 expressing stromal cells (Miyake et al., 1991; Arroyo et al., 1999). The ß1 and ß2 integrins have been observed to mediate interactions of B-CLL cells with bone marrow stromal cells and inhibit spontaneous apoptosis *in vitro* (Lagneaux et al., 1998). ß7 has also been observed to be expressed in B-CLL cells (Vincent et al., 1996).

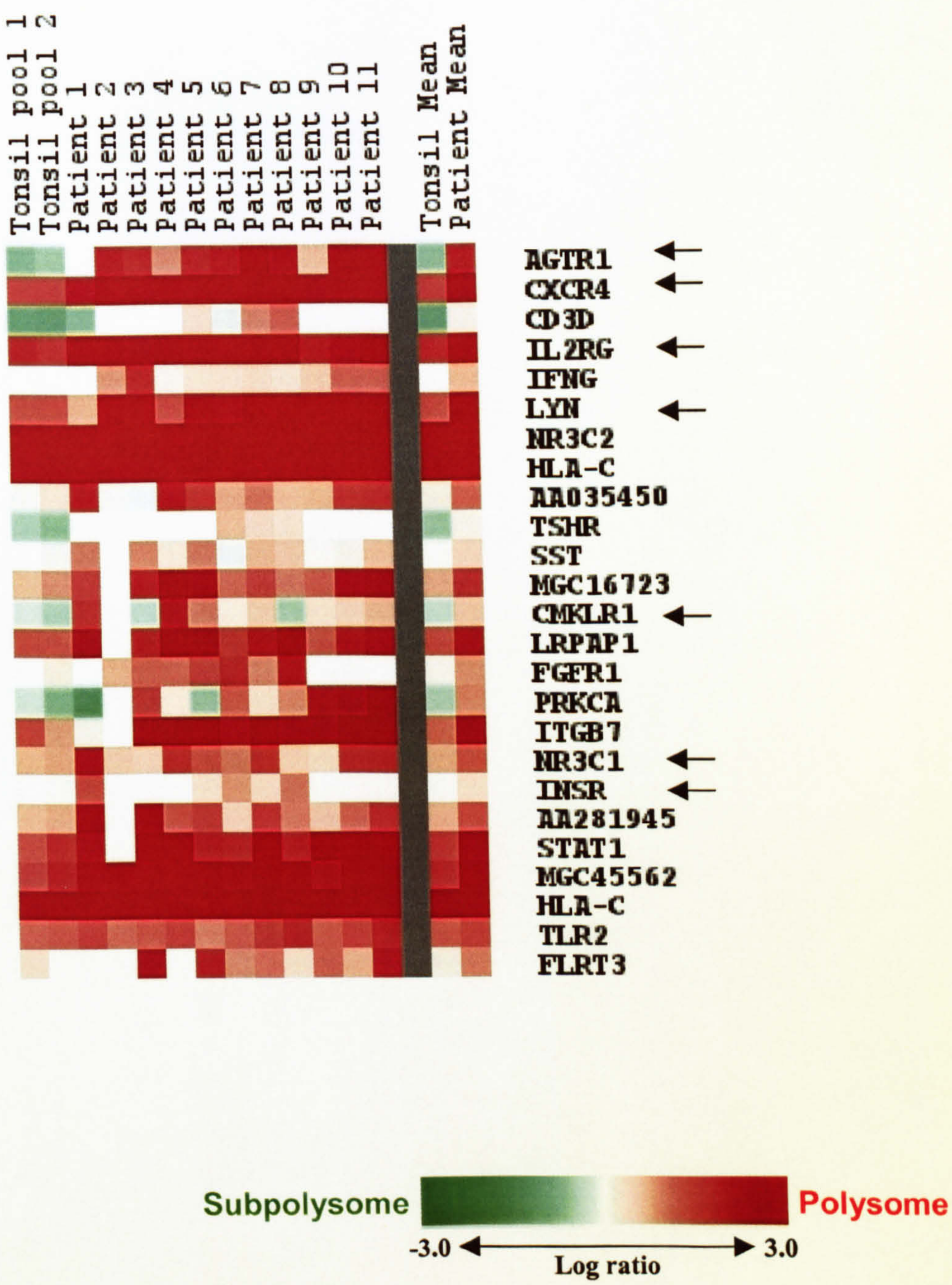


Figure 4.6 Receptor genes with increased polysome association in B-CLL cells, when compared to tonsil CD19+ B cells. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated. Receptors which may be involved in survival signalling are highlighted by arrows.

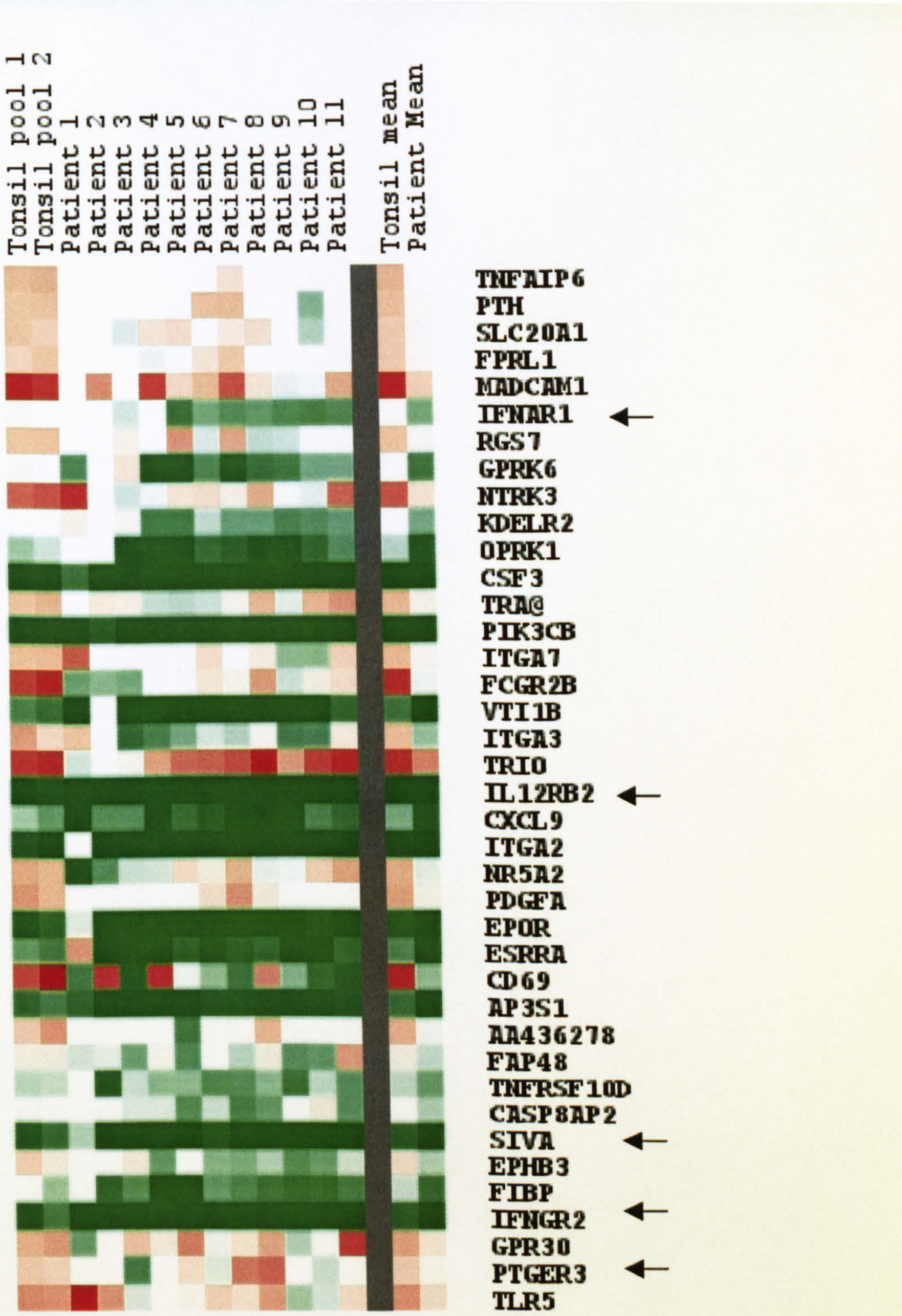


Figure 4.7 Receptor genes with decreased polysome association in B-CLL cells, when compared to tonsil CD19+ B cells. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated. Receptors which may be involved in survival signalling are highlighted by arrows.

AGTR1 (angiotensin II receptor type 1) was observed to have increased polysome association in B-CLL cells (Figure 4.6 and Appendix 3.6). It is a G protein coupled receptor used by the hormone angiotensin II (ang II) (Timmermans et al., 1993). Ang II has a major role in regulating blood pressure as it causes constriction of blood vessels (Sadoshima and Izumo 1993; Sadoshima et al., 1993). It also has effects on a wide range of other systems including the endocrine and nervous systems (Peach 1977; Peach and Dostal, 1990). AGTR1 has been shown to mediate the actions most associated with ang II however there is a second type II receptor but its role is less well defined. There is increasing evidence for a role for ang II in regulation of cell growth, death and differentiation in various cell types. Ang II induces growth in vascular smooth muscle cells, which occurs through an increase in protein synthesis (Owens, 1989). There is evidence that this could be due to phosphorylation of eIF4E, 4EBP1 and p70S6K and decreased phosphorylation of eEF2 (Servant et al., 1996; Giasson and Meloche, 1995; Sadoschima and Izumo, 1995; Boluyt et al., 1997; Everrett et al., 2001; Ishida et al., 2003). Ang II may also play a role in regulating apoptosis. It was shown to decrease NMDA receptor mediated cell death in neuronal cells (Schelman et al., 2004). This action was found to be mediated by the type 1 or type 2 receptor and also prevented the reduction in Bcl2, which occurs in this death pathway. However it has also been observed that ang II can induce apoptosis in human endothelial cells, mediated by both the type 1 and type 2 receptors (Dimmeler et al., 1997). Ang II may also have a role in inflammation. Ang II, acting through the type 1 receptor, has been reported to stimulate the production of a number of inflammatory mediators such as TNFα, TGFß and MCP1 (Klahr and Morrissey, 1998) and has been shown to activate NF-κB, a key transcription factor in inflammation (Müller et al., 2000; Ruiz-Ortega et al., 2000; Kranzhöfer et al., 1999; Wolf et al., 2002). There is much evidence for translation control of AGTR1 expression. The 5'UTR of AGTR1 mRNA contains complex secondary structure and two uORFs which are inhibitory to cap-dependent translation initiation (Curnow et al., 1995; Martin et al., 2001a, 2001b). The secondary structure has been shown to contain an IRES element which can be utilised for efficient translation (Martin et al., 2003). Oestrogen has also been shown to modulate the translation of AGTR1, acting through elements in the 5'UTR (Wu et al., 2003).

There are a number of proteins involved in cell signalling observed to have differential polysome association in B-CLL (Figure 4.8 and Appendix 3.7, Figure 4.9 and Appendix 4.7), which could affect downstream signal transduction and the responses to signals

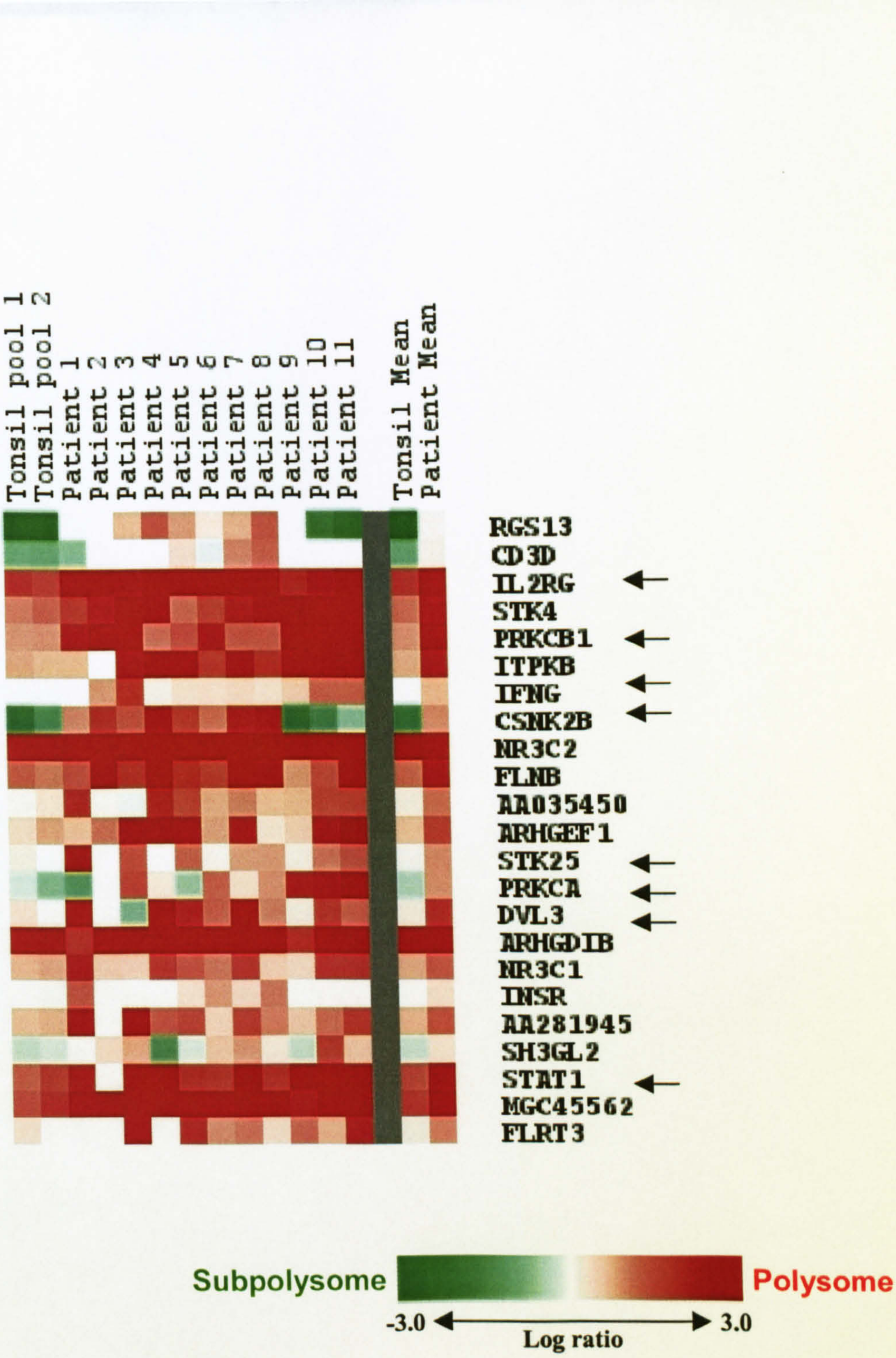


Figure 4.8 Cell signalling genes with increased polysome association in B-CLL cells, when compared to tonsil CD19+ B cells. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated. Genes associated with survival signalling are highlighted by arrows.

Figure 4.9 Cell signalling genes with decreased polysome association in B-CLL cells, when compared to tonsil CD19+ B cells. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated. Genes associated with survival signalling are highlighted by arrows.

received by cells. IFNγ (interferon gamma) was observed to have increased polysome association in B-CLL cells (Figure 4.8 and Appendix 3.7). It has anti-proliferative and immunoregulatory functions and is normally produced by activated lymphocytes, in particular type 1 T cells and natural killer cells (Billiau and Vanderbroek, 2001). IFNγ has been shown to increase survival of B-CLL cells *in vitro* plus has been detected in the serum of B-CLL patients (Buschle et al., 1993). The receptor for IFNγ has two subunits, R1 and R2. It has previously been found to be up-regulated in B-CLL cells, and T cells from B-CLL patients were shown to produce increased amounts (Zaki et al., 1998; Reyes et al., 1998). However the R2 subunit (IFNGR2) was observed to have decreased polysome association in B-CLL cells by the microarray analysis (Figure 4.7 and Appendix 4.6), which is in contrast to the previous data.

Lyn (v-yes-1 Yamaguchi sarcoma viral related oncogene homolog) was observed to have increased polysome association in B-CLL cells (Figure 4.6 and Appendix 3.6). It is a member of the src family of protein tyrosine kinases and is involved in BCR signalling. Upon stimulation of the BCR Lyn is recruited to the receptor where it phosphorylates immunoreceptor tyrosine-based activation motifs (ITAMs) of CD79a and CD79b, the intracellular components of the BCR (Nagai et al., 1995; Johnson et al., 1995). Another protein tyrosine kinase, Syk can then bind to these domains leading to autophosphorylation and further propagation of BCR signalling by further tyrosine phosphorylation (Turner et al., 2000). Lyn is a key molecule in BCR receptor signalling due to its direct binding at the plasma membrane and is therefore involved in regulating downstream events. Lyn is also involved in BCR-independent signalling. For example, Lyn has been observed to be involved in Fas-dependent apoptosis (Wang et al., 1996) and in MHC class II signalling (Lang et al., 2001).

STAT1 (signal transducer and activator of transcription 1) also had increased polysome association in B-CLL cells (Figure 4.8 and Appendix 3.7). It is a latent cytoplasmic transcription factor which, upon stimulation, translocates to the nucleus and regulates transcription of specific genes. STAT1 activation is associated with growth arrest and apoptosis, which may be mediated by STAT1 induction of caspase 1 and p21 expression (Chin et al., 1996; Kumar et al., 1997), and this aberrant signalling can lead to transformation and oncogenesis (Bowman et al., 2000). STAT1 has also been observed to be constitutively phosphorylated on serine residues in B-CLL cells (Frank et al., 1997).

Serine phosphorylated STAT1 molecules have been shown to be localised in the cytoplasm; tyrosine phosphorylation is required for translocation to the nucleus. STAT1 has been observed to associate with other cytoplasmic proteins, therefore this may be the case in B-CLL cells (Lackmann et al., 1998; Usacheva et al., 2001; Battle and Frank, 2003). STAT1 is also involved in the downstream signalling from some of the receptors shown to have increased polysome association in B-CLL cells by the microarray analysis, including AGTR1 and IFNγ (Marrero et al., 1995; Stark et al., 1998; O'Shea et al., 2002; Levy and Darnell, 2002; Shuai and Liu, 2003). There is also evidence that STAT1 can be translationally regulated as c-Cbl has been observed to repress its translation (Blesofsky et al., 2001).

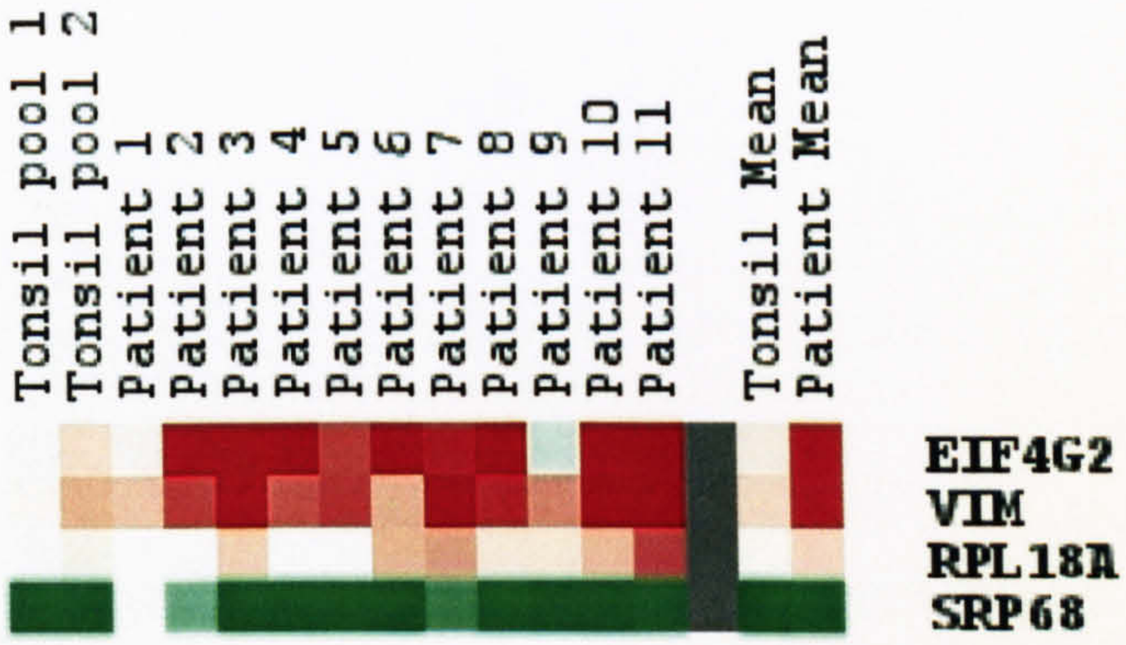
PIAS1 (protein inhibitor of activated STATs 1) had decreased polysome association in B-CLL cells (Figure 4.9 and Appendix 4.7). PIAS1 inhibits STAT1 DNA binding and subsequent gene activation (Arora et al., 2003). Therefore, a reduction in PIAS1 may contribute to potential increased STAT1 signalling in B-CLL cells. A recent study suggests that PIAS1 selectively regulates a subset of STAT1 responsive genes, which includes IFNγ and IFNß responsive genes (Liu et al., 2004), indicating a role for PIAS1 in immune response. Downregulation in B-CLL cells could therefore potentially enhance IFNγ signalling.

Protein kinase C (PKC) isoforms α and ß were observed to have increased polysome association in B-CLL cells (Figure 4.8 and Appendix 3.7). These are both classical isoforms of PKC that can be regulated by calcium, diaglycerol (DAG) and phospholipids (Mellor and Parker, 1998; Nishizuka, 2003), and have been implicated in numerous cellular processes, including cell growth and apoptosis. PKC is involved in the signalling pathways of receptors identified in the microarray analysis such as AGTR1 (Karim et al., 1995; Greenland and Mukhopadhyay, 2004). It also has an important role in immune response and has been identified as a component of antigen receptor signalling (Tan and Parker, 2003). Activation of PKC in lymphocytes can cause morphological changes and affect integrin mediated cell adhesion or migration (Hogg et al., 2003). It has also been shown to activate NF-κB (Tan and Parker, 2003). There is some evidence that PKC may be involved in B-CLL survival, since inhibition of PKC in B-CLL cells caused apoptosis (Barragan et al., 2002). The downstream pathway may involve activation of NF-κB and increased expression of $BclX_L$, Mcl-1 and XIAP (Furman et al., 2000; Kitada et al., 1999).

CSNK2B (casein kinase II beta) was also found to have increased polysome association in B-CLL cells (Figure 4.8 and Appendix 3.7). This is the regulatory subunit of casein kinase II, a kinase involved in a diverse range of cellular processes that has hundreds of substrates (reviewed (Litchfield, 2003; Meggio and Pinna, 2003). There is evidence to suggest that casein kinase II may be required for cell viability. Disruption of CSNK2B in mice and RNAi mediated knockdown in C. *elegans* led to defects in development (Ahmed et al., 2002; Fraser et al., 2000). Casein kinase II may also have an anti-apoptotic role. Increased expression of casein kinase II was found to protect cells from drug induced apoptosis and phosphorylation of certain proteins by the kinase protected them from caspase mediated cleavage (Guo et al., 2001; Desagher et al., 2001; Yin et al., 2001; Ruzzene et al., 2002; Li et al., 2002). Increased casein kinase II expression has been observed in a number of cancers which may result in enhanced cell survival (Landesman-Bollag et al., 2001; Yenice et al., 1994; Dayamakin et al., 1994; Faust et al., 1996). A number of translation factors are phosphorylation targets of casein kinase II and it has been suggested that this may be important for maintaining some components of the translation machinery in a functional confirmation (Pinna, 1990; Meggio and Pinna, 2003).

4.6 Translation factors

eIF4GI was observed to have decreased polysome association in B-CLL cells (Figure 4.11 and Appendix 4.8), whereas eIF4G2 was observed to have increased polysome association (Figure 4.10 and Appendix 3.8). eIF4G2 is not the functional isoform eIF4GII, but an alternative name for DAP5 (death associated protein 5). All three proteins are part of the eIF4G family, which share homology in a domain that binds eIF4A and eIF3 (Gingras et al, 1999). eIF4GI and eIF4GII support cap-dependent translation, whilst DAP5 (also known as NAT1 or p97) is homologous to the C-terminal two thirds of eIF4GI so lacks the cap-binding section and cannot support cap-dependent translation (Hentze, 1997; Morley et al., 1997; Gingras et al., 1999; Keiper et al., 1999). Overexpression of DAP5 has been shown to inhibit both cap-dependent and cap-independent translation consequently leading to an inhibition of proliferation (Yammanaka et al., 1997; Imataka et al., 1997). This would suggest that the expression levels observed for DAP5 would fit with the growth suppressed phenotype of B-CLL cells. There is also evidence that reveals DAP5 may play a role in IRES mediated translation, where cap binding is not required. An IRES element has been previously been identified for DAP5 (Henis-Korenblit et al., 2000). During apoptosis DAP5 is cleaved to yield a p86 isoform, which has been shown to enhance the translation



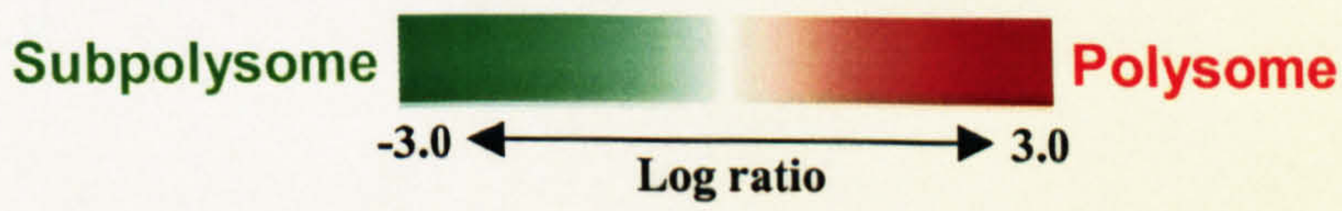


Figure 4.10 Translation associated genes with increased polysome association in B-CLL cells, when compared to tonsil CD19+ B cells. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated.

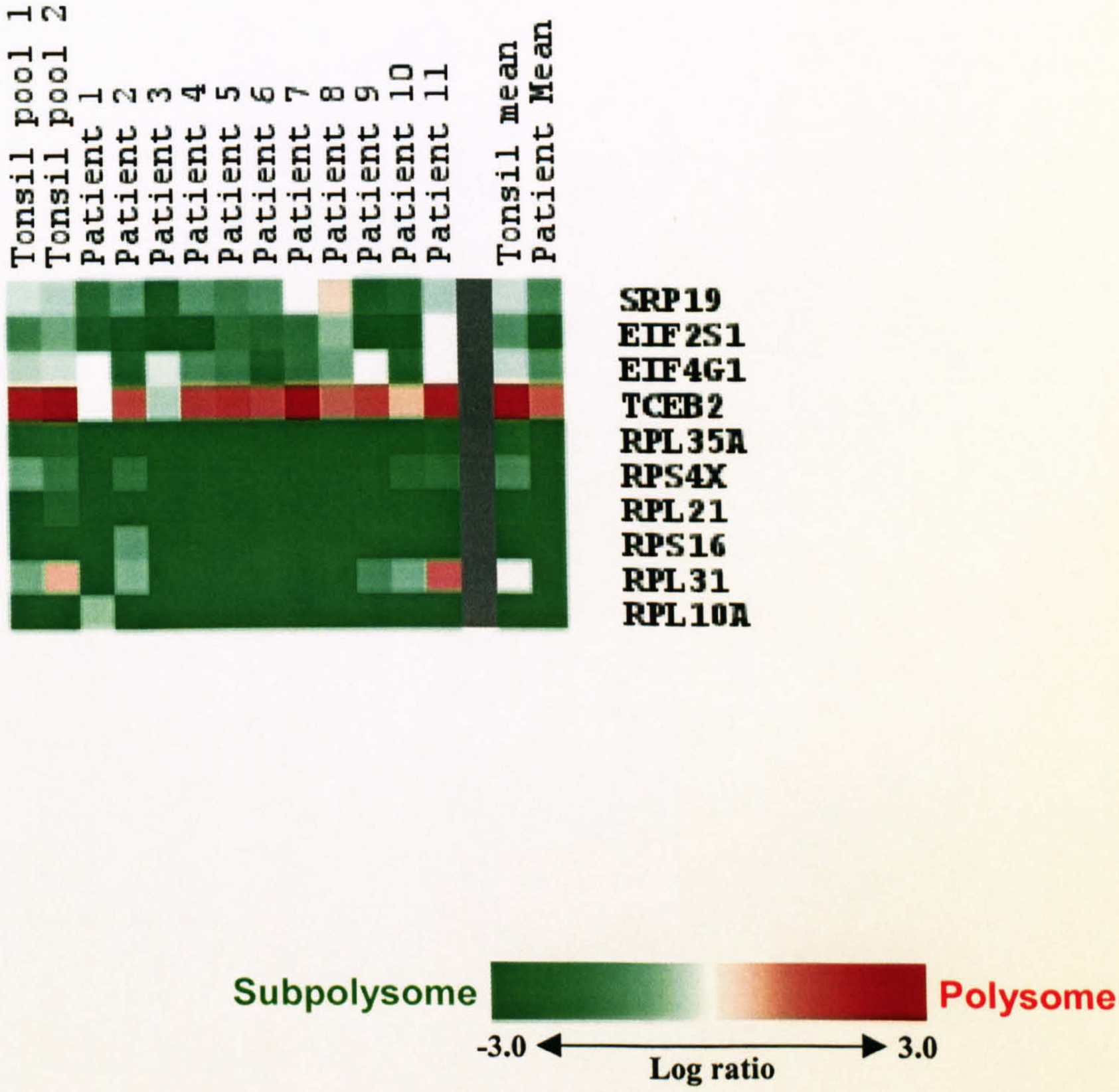


Figure 4.11 Translation associated genes with decreased polysome association in B-CLL cells, when compared to tonsil CD19+ B cells. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated.

from its own IRES as well as those of other death associated proteins, c-Myc, Apaf-1 and XIAP (Henis-Korenblit et al 2000, 2002).

A number of ribosomal proteins show differential polysome association for B-CLL cells (Figure 4.10 and Appendix 3.8, Figure 4.11 and Appendix 4.8). Ribosomal proteins are components of the ribosome that can function as RNA chaperones during the assembly of the ribosome subunits, in stabilisation of important domains or may coordinate interactions with the mRNA or translation factors (Maguire and Zimmerman, 2001; Ramakrishnan, 2002). The synthesis of ribosomal proteins is usually a coordinated process which produces equimolar amounts of each protein (Warner, 1999). They are part of the 5'TOP class of proteins whose regulation is discussed in section 1.4.4. Expression of specific ribosomal proteins can be regulated separately under specific conditions however. Aberrant expression of ribosomal proteins has been observed in cancer cell lines and tumours (Chung et al., 2002; Karan et al., 2002; Lopez et al., 2002), which can lead to defects in ribosomes and affect translation. Gene targeting in yeast shows that defects in just one ribosomal protein can lead to defective protein synthesis (Rotenberg et al 1988). In addition drosophila with ribosomal protein mutations, known as minute flies, are characterised by a reduced body size, diminished fertility and recessive lethality (Kongsuwan et al, 1985). Further analysis revealed these flies to contain a reduced number of ribosomes resulting in lower levels of protein synthesis (Kay and Jacobs, 1987). It is possible that changes in the level of specific ribosomal proteins in B-CLL cells could cause ribosome defects and reduce the rate of protein synthesis.

4.7 Semi-quantitative RTPCR analysis confirms the microarray data obtained

The data produced from the microarray analysis was confirmed using semi-quantitative RTPCR. PCR was performed for differing numbers of cycles (ranging from 4 to 24), using cDNA representing polysome and subpolysome mRNA. The products were then blotted and probed with specific radioactive probes, detected by phosphoimager analysis, so that a ratio of polysome to subpolysome mRNA could be calculated. This was performed for B-CLL cells and tonsil CD19+ B cells and the data compared for each gene to identify differences in polysome association between the two cell types. These results were then compared to the microarray datum for each gene. The analysis was initially performed for actin, which was observed to have a similar polysome association in B-CLL cells and tonsil CD19+ B cells by Northern analysis (Figure 3.8). The semi-quantitative RTPCR analysis

agreed with the Northern data (Figure 4.12), suggesting that this technique was an accurate method to use for determination of percentage polysome association. This was then subsequently used to analyse the polysome association for a selection of genes observed to have differential polysome association in the microarray analysis. These were UCP2, BTG1. WEE1 and CSNK2B, which were all found to have increased polysome association in B-CLL cells, and cyclin D3 which had decreased polysome association. Those mRNAs shown to have increased polysome association in B-CLL cells by microarray analysis, were also found to have increased polysome association by semi-quantitative RTPCR analysis and vice versa for cyclin D3 (Figure 4.12). Therefore this suggests that the data produced by the microarray analysis was accurate.

4.8 Analysis of cyclin D protein levels

Cyclin D3 was identified in the microarray analysis as having decreased polysome association in B-CLL cells (Figure 4.5), and this was confirmed by semi-quantitative RTPCR analysis (Figure 4.12). There are three isoforms of cyclin D; D1, D2 and D3, which are involved in promoting the G1/S cell cycle transition. They bind to and stimulate the catalytic activity of CDK4 or CDK6, which then phosphorylate retinoblastoma protein (Rb) leading to its release from the transcription factor E2-F. E2-F is then free to activate components of the DNA replication machinery committing the cell to S phase (Weinberg, 1995; Muller and Helin, 2000) (Figure 1.1). The three isoforms are thought to be functionally redundant in this activity, however not all cells express all three and they may have specific roles, depending on abundance and cell type. Western analysis was performed to determine protein levels of D type cyclins in B-CLL cells (Figure 4.13). Cyclin D3 protein levels were observed to be reduced in the B-CLL samples compared to that in tonsil CD19+ B cells, agreeing with the microarray data. Cyclin D1 levels were the same in B-CLL cells as for tonsil CD19+ B cells, but cyclin D2 levels were increased. Some previous studies have analysed cyclin D levels in B-CLL cells. Cyclin D2 has previously been observed to be overexpressed in B-CLL cells by a number of studies (Delmer et al., 1995; Woloweic et al., 2001), agreeing with the Western analysis performed. The data for cyclin D3 is more variable. Some studies have found no detectable cyclin D3 in B-CLL cells (Delmer et al., 1995), whilst others have found that some cyclin D3 is present (Suzuki et al., 1999; Woloweic et al., 2001). The microarray and protein datum reported here agrees with a reduced level of cyclin D3. If this was the case, it would correlate with the quiescent phenotype observed for B-CLL cells (Andreeff et al., 1980;

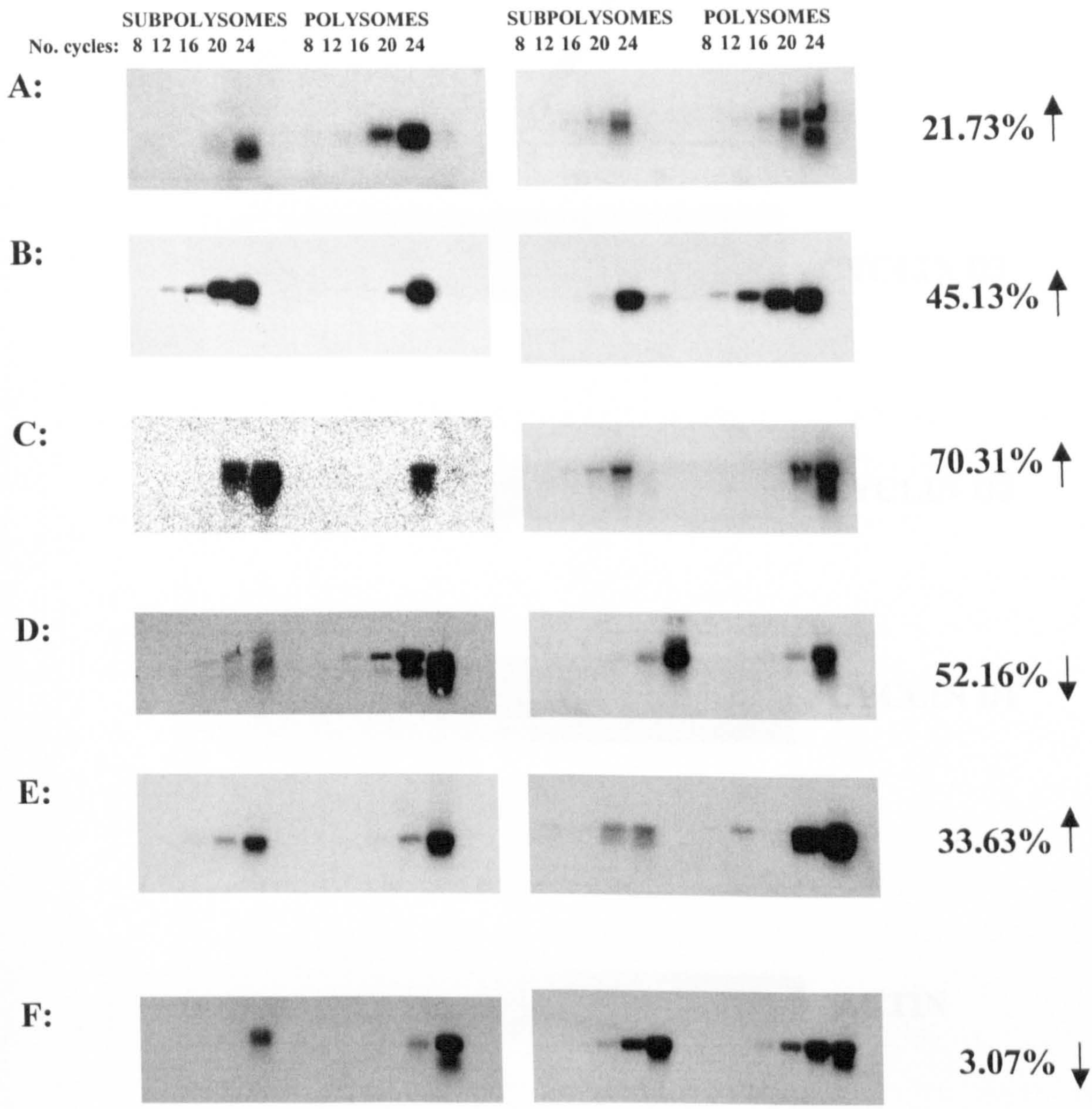


Figure 4.12 Confirmation of microarray results using semi-quantitative RTPCR. cDNA representing subpolysomes and polysomes was synthesised and then used for PCR reactions for 8, 12, 16, 20 and 24 cycles. Southern analysis was performed on the PCR products to determine the distribution of mRNA between the subpolysome and polysome pools, for B-CLL cells and tonsil CD19+ B cells. The percentage polysome association for the mRNA was then compared between the two cell types. This was performed for UCP2 (A-patient 1), BTG1 (B-patient 1), WEE1 (C-patient 4), Cyclin D3 (D-patient 4), CSNK2B (E-patient 1) and actin (F-patient 1). For all genes tested, the difference in percentage polysome association between the two cell types agreed with the results obtained in the microarray analysis. Analysis for each gene was performed once and with one patient.

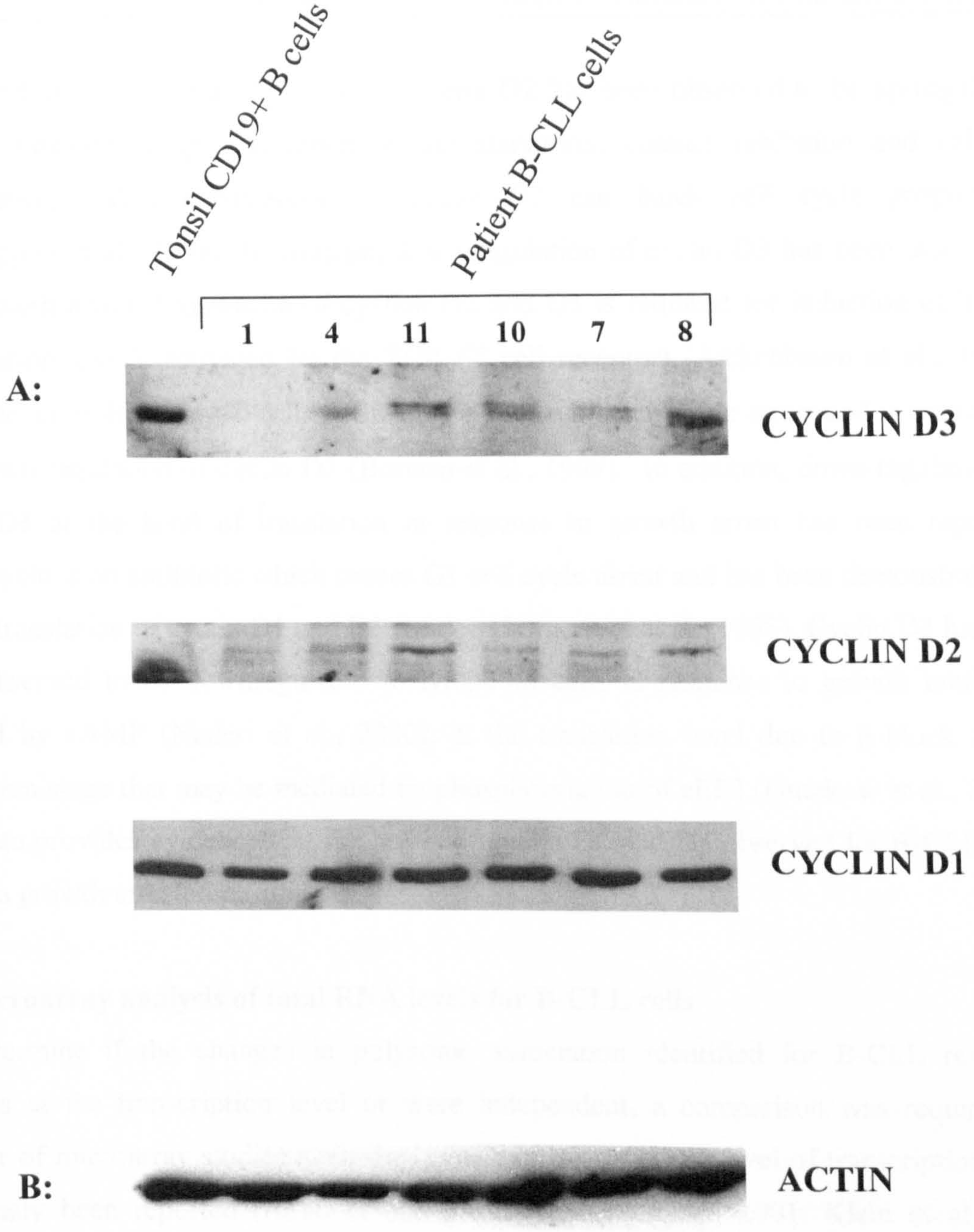


Figure 4.13 Western analysis of cyclin D levels in B-CLL cells. Cell lysates were made from B-CLL cells and tonsil CD19+ B cells and separated by SDS PAGE. The gels were then immunoblotted and probed with specific antibodies to cyclin D1, D2 and D3 (A), and actin (B). Cyclin D3 protein levels were observed to be lower in B-CLL cells that tonsil CD19+ B cells, which agrees with the microarray results. Cyclin D2 levels were observed to be higher and cyclin D1 the same for both cell types. Each Western was performed once.

Caligaris-Cappio and Hamblin, 1999). Cyclin D2 has been observed to be up-regulated under conditions of growth arrest; serum starvation, contact inhibition and cellular scenescence, and overexpression of cyclin D2 can block cell cycle progression (Meyyappan et al., 1998). In contrast, down-regulation of cyclin D3 has been associated with growth arrest. Expression of cyclins D2 and D3 is required for induction of T cell proliferation and is activated by the TCR (T cell receptor) (Ajchenbaum et al., 1993). However, in proliferating T cells, TCR activation causes cell cycle arrest and is associated with down-regulation of cyclin D3 (Boonen et al., 1999). In addition, down-regulation of cyclin D3 at the level of translation in response to growth arrest has been reported. Herbimycin is an antibiotic which causes G1 cell cycle arrest and has been demonstrated to inhibit translation of cyclin D1 and D3 (Muise-Helmericks et al., 1998). Cyclin D3 has also been observed to be downregulated in lymphoid cells in response to growth inhibition induced by cAMP (Naderi et al., 2000), at the translation level due to a block at the elongation stage that may be mediated by phosphorylation of eEF2 (Gutzkow et al., 2003). This data provides evidence that the levels of cyclin D2 and D3 observed for B-CLL cells reflect a growth arrested phenotype.

4.9 Microarray analysis of total RNA levels for B-CLL cells

To determine if the changes in polysome association identified for B-CLL reflected changes at the transcription level or were independent, a comparison was required. A number of microarray studies analysing gene expression at the level of transcription have previously been reported (Aalto et al., 2001; Stratowa et al., 2001; Klein et al 2001; Rosenwald et al 2001; Jelinek et al 2003). However, a small microarray study was performed using the same microarray slides as used for the translation analysis so a direct comparison could be performed. Total RNA was isolated from all 11 B-CLL and all tonsil CD19+ B cell samples and then that for each cell type pooled. Fluorescently labelled cDNA representing each pool was synthesised, labelling with cy3 for one cell type and cy5 for the other, and hybridised to a microarray. This was performed in triplicate, reversing the cy dye labelling for one of the microarrays. A direct comparison of total RNA levels for each gene could be calculated. The fluorescence intensities obtained from the microarray scans were analysed in GenePix 3.0 and expressed as a ratio of cy3/cy5 for each gene on the microarray. The data for each microarray was then normalised and a T-test performed for the three hybridisations with a significance p value of 0.05. 514 genes were identified

with significantly different levels of total RNA in B-CLL cells compared to tonsil CD19+ B cells. 157 had increased levels for B-CLL (Appendix 5) and 357 decreased (Appendix 6).

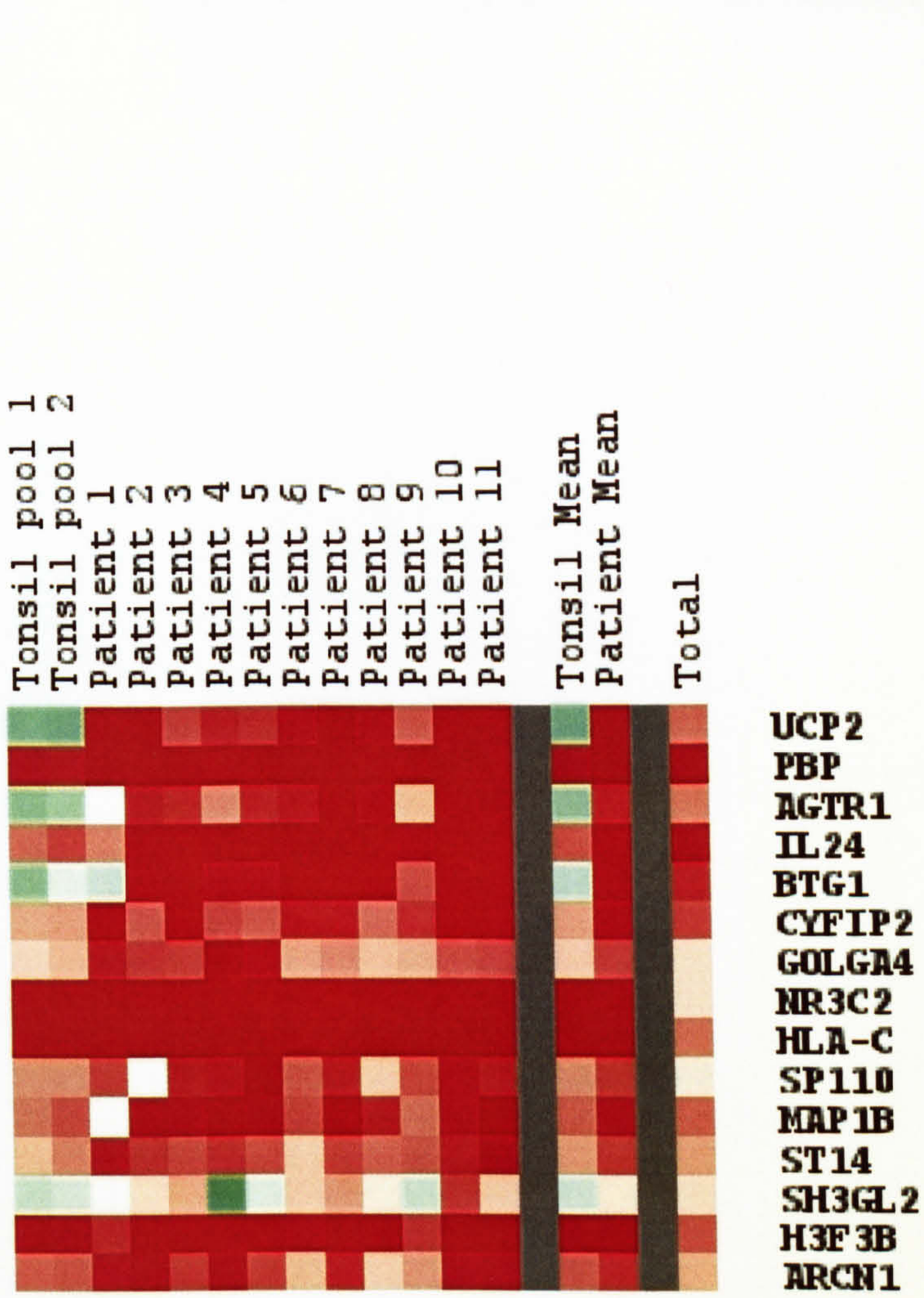
4.10 Comparison of transcription and translation data

The microarray data obtained for B-CLL, analysing changes in gene expression at the level of transcription or translation was compared. 16 genes were observed to have both up-regulated transcription and translation (Figure 4.14 and Appendix 7.1), and 35 down-regulated transcription and translation (Figure 4.15 and Appendix 7.2), which was only a small proportion of the total genes identified with a significant change in gene expression at either level. This suggests that the changes identified at each level of gene expression were relatively independent of each other. It also highlights the importance of translational regulation and its independence from regulation at the level of transcription rather than following the same pattern. However, a number of the genes found up-regulated at both levels of expression were observed to have some of the largest increases in polysome association in B-CLL, including AGTR1, BTG1, UCP2 and IL24 (Figure 4.14). This may reflect a highly increased expression level overall for these genes produced by up-regulation at multiple stages in the gene expression pathway.

There are also a number of genes which show differential expression between transcription and translation. 13 genes were found to have up-regulated transcription but down-regulated translation (Figure 4.16 and Appendix 7.3) and 13 genes had down-regulated transcription but up-regulated translation (Figure 4.17 and Appendix 7.4). This highlights that changes observed at the level of transcription may not necessarily reflect what occurs at the protein level, and the importance of analysing gene expression at multiple levels of the gene expression pathway.

4.11 Discussion

cDNA microarray analysis was performed for B-CLL cells to determine a translation profile, producing data which correlated well with the biology of the disease. The microarray analysis determined the amount of polysome associated message for each gene, as translated messages were assumed to be those with multiple ribosomes attached. This was performed for 11 B-CLL patients, then the data compared to control cells so that differences in polysome association, and therefore translation, could be identified. The B-CLL cell data was first compared to that for GM1953 cells. 1982 genes were identified



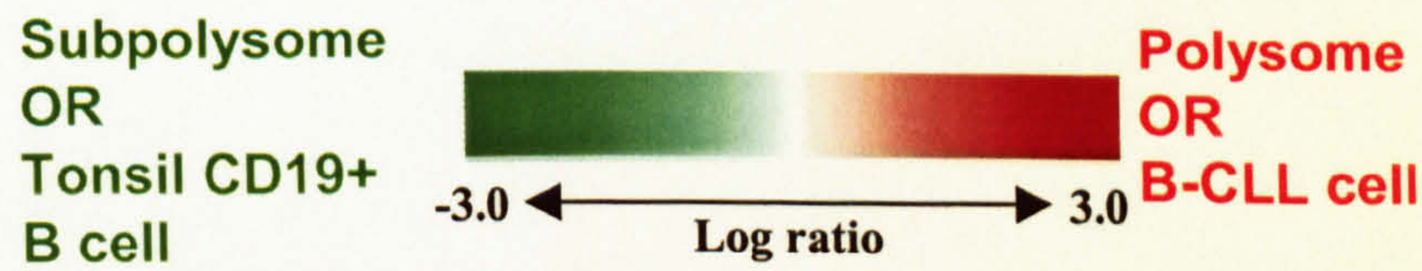


Figure 4.14 Genes identified with up-regulated transcription and translation. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated. The total column represents the transcription data. A red square means there was higher mRNA levels in B-CLL cells than tonsil CD19+ B cells and green lower.

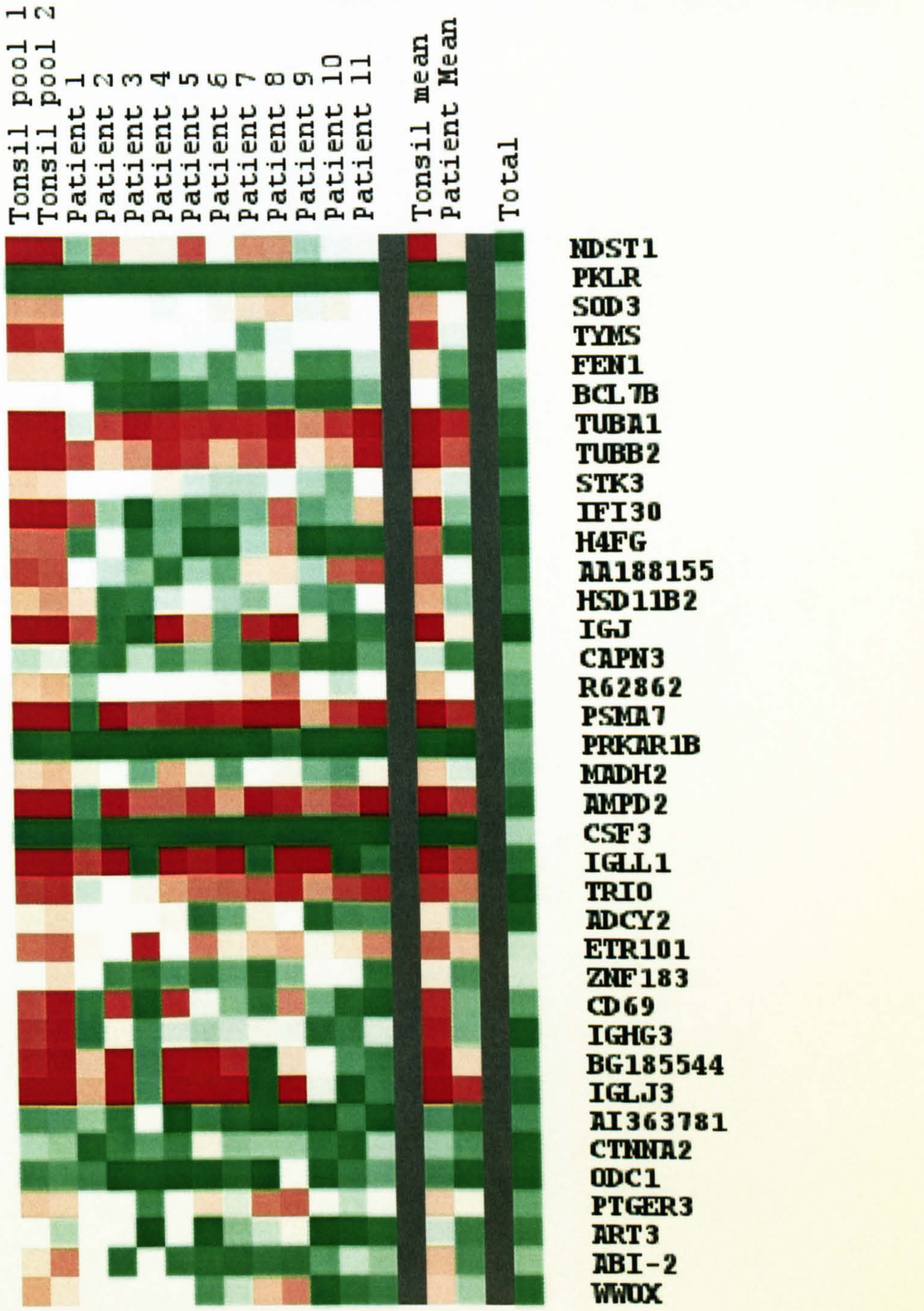


Figure 4.15 Genes identified with down-regulated transcription and translation. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated. The total column represents the transcription data. A red square means there was higher mRNA levels in B-CLL cells than tonsil CD19+ B cells and green lower.

Figure 4.16 Genes identified with up-regulated transcription but down-regulated translation. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated. The total column represents the transcription data. A red square means there was higher mRNA levels in B-CLL cells than tonsil CD19+ B cells and green lower.

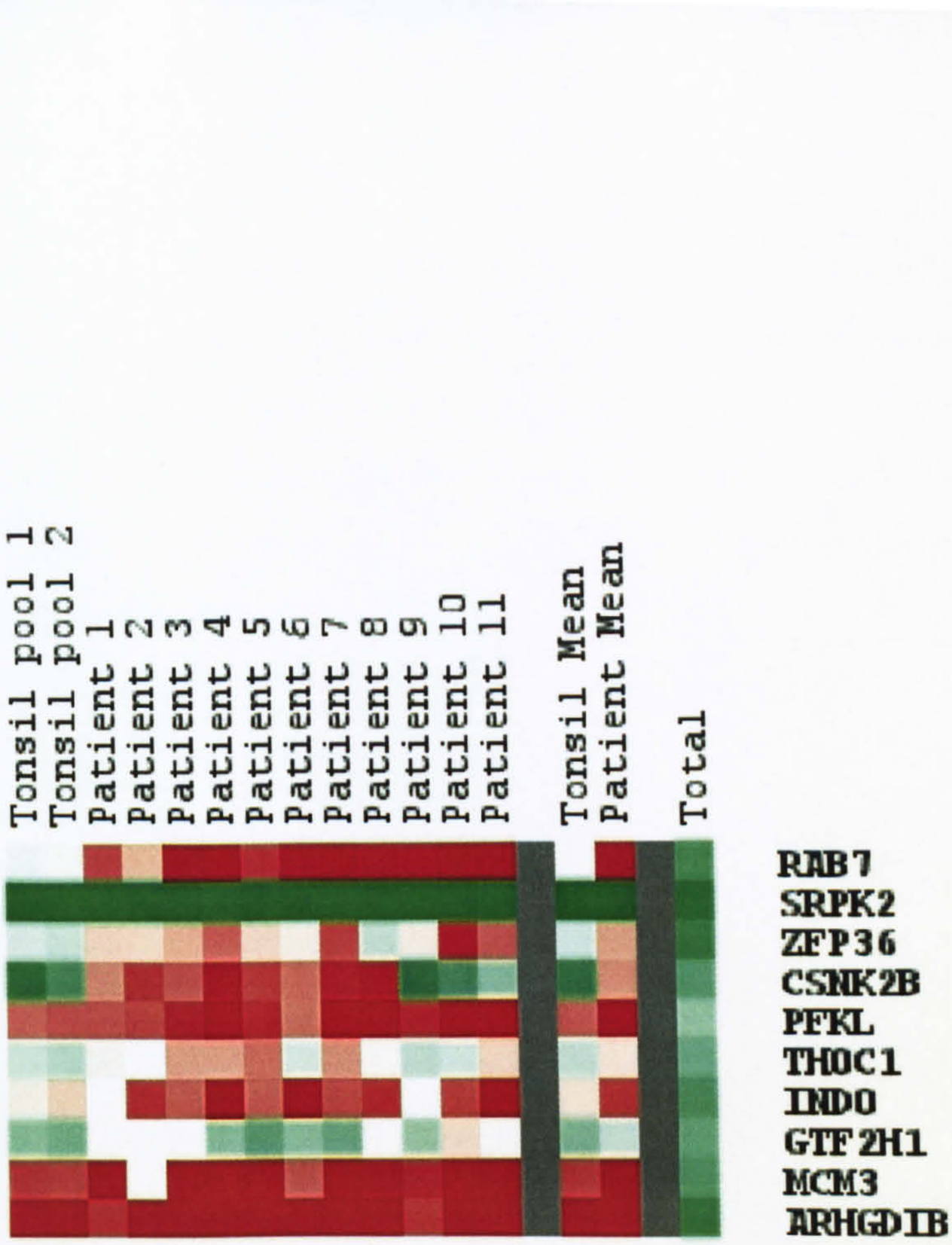


Figure 4.17 Genes identified with down-regulated transcription but up-regulated translation. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated. The total column represents the transcription data. A red square means there was higher mRNA levels in B-CLL cells than tonsil CD19+ B cells and green lower.

with differential polysome association, 909 with increased association and 1073 decreased. This was a large number of genes to be differentially expressed and probably reflected differences due to the immortalised phentotype of GM1953 cells, rather than being significant to the biology of B-CLL. Therefore, GM1963 cells were not a suitable control to use for comparison to B-CLL cells. Instead B-CLL data was compared to data for tonsil CD19+ B cells. 493 genes were identified with differential polysome association, 181 with increased association and 312 decreased. This was a more reasonable number of genes to be observed to have differential expression. Tonsil CD19+ B cells are primary and therefore make a more suitable control, so any differences identified were more likely to be relevant to the biology of the disease. However, as they are not the cell of origin for B-CLL, the changes identified may reflect this rather than being relevant to tumorigenesis.

Initial analysis of the data reveals that this was the case. A number of proteins which may have anti-apoptotic activity were observed to have increased polysome association, such as Bcl2, IL24 and UCP2, and pro-apoptotic proteins such as PDCD2, PDCD5 and SIVA were observed to have decreased polysome association. Therefore the change in level of these proteins could contribute to the defective apoptosis observed for B-CLL. The observation that B-CLL cells are quiescent and found in the G0/G1 phase of the cell cycle is also thought to be important in B-CLL tumorigenesis (Andreeff et al., 1980; Caligaris-Cappio and Hamblin, 1999). Thus the increased polysome association of BTG1, an anti-proliferative factor usually expressed in the G0 phase of the cell cycle may be of significance. Up-regulation of WEE1 may also be important, however, this protein is associated with the prevention of early entry into mitosis, therefore how this would effect B-CLL is unclear. Cyclin D3 was identified to have decreased polysome association in B-CLL cells. This was also observed at the protein level. Reduced cyclin D3 levels have been reported in arrested cells previously (Boonen et al., 1999; Muise-Helmericks et al., 1998; Naderi et al., 2000). Cyclin D2 protein levels were also observed to be increased, which has been reported previously (Delmer et al., 1995; Woloweic et al., 2001) and been found to be increased under conditions of cell cycle arrest (Meyyappan et al., 1998). A number of receptors, or proteins involved with cell signalling may be involved in the signal transduction of survival signals *in vivo*. CXCR4 was observed to have increased polysome association in B-CLL cells and has previously been suggested to play a role in B-CLL cell survival. Interleukins such as IL4 have also been suggested to affect B-CLL survival. Receptors for a number of interleukins contain IL2RG, the common gamma chain, as one

component and this was observed to have increased polysome association in B-CLL cells. Alterations in the expression of integrins may also be significant. Other receptors with increased polysome association, such as AGTR1, may also be involved. An alteration in proteins involved in the signal transduction from these receptors, such as STAT1, PKC and casein kinase II, may also affect the downstream response observed. Changes in the translation machinery may alter the rate of translation observed. Aberrant expression of ribosomal proteins has previously been observed in cancer and can lead to ribosomal defects, therefore those ribosomal proteins observed to have a change in polysome association in B-CLL cells may affect the rate of translation. The increased polysome association of DAP5 and decreased association of eIF4GI could also be of significance. DAP5 generally represses translation, although there is evidence it could support specific cap-independent translation (Henis-Korenblit et al 2000, 2002). Overall, evidence was provided, that a number of the genes identified with differential polysome association in B-CLL cells may be important to the biology of the disease. It also shows that changes in gene expression at the level of translation are important in tumorigenesis.

The microarray data alone does not provide conclusive evidence that the changes observed will be significant in B-CLL. Initially, semi-quantitative RTPCR has been performed to determine if the polysome association observed in B-CLL cells and tonsil CD19+ B cells for a selection of genes was correct. For all genes analysed, the difference in polysome association between B-CLL cells and tonsil CD19+ B cells was observed to be the same as that observed in the microarray analysis. Therefore, this suggested that all the results produced in the microarray analysis would also be correct, although direct confirmation would be required for any gene if further analysis was to be performed.

The differences observed in polysome association could have just reflected what was occurring at the level of transcription. Therefore, microarray analysis was performed using total RNA from B-CLL cells and tonsil CD19+ B cells for comparison with the translation data. Only a small number of genes were identified to have an increase or decrease at both levels of gene expression, suggesting that regulation at each stage was independent of the other. An increase or decrease in both transcription and translation may reflect a highly up or down-regulated level of expression. There were also a number of genes which were up-regulated at transcription but had decreased polysome association, or vice versa. This

highlights the importance of studying gene expression at multiple steps in the pathway otherwise an inaccurate profile of gene expression may be obtained.

Chapter 5

Functional and mechanistic analysis of translation in B-CLL cells

5.1 Introduction

There are a number of ways by which translation can be regulated, as discussed in section 1.4, and some of these mechanisms could be deregulated in B-CLL. B-CLL cells were observed to have a reduced level of translation (chapter 3) so it is possible that, as an alternative mechanism of initiation, internal ribosome entry might allow increased expression of specific messages. It is also important to determine what effect changes in gene expression have on the biology of the disease. Specific mRNAs shown to be de regulated may have an important role in the biology of B-CLL and could be utilised as targets for new treatments. B-CLL cells do not apoptose *in vivo* yet rapidly die *in vitro* (Collins et al., 1989; MacFarlane et al., 2002). They are thought to require signals from the *in vivo* microenvironment for their survival, so receptors for such a signal might be up-regulated in B-CLL cells.

Evidence is provided that IRES elements are found in the 5'UTRs of three mRNAs, BTG1, UCP2 and CSNK2B, which were shown to have significantly increased polysome association in B-CLL cells by the microarray analysis performed. Their IRES activity was also observed to be maintained under conditions of serum starvation. Moreover angiotensin II, whose receptor AGTR1 was also shown have increased polysome association in B-CLL cells by the microarray analysis, plus has previously been observed to contain an IRES in its mRNA (Martin et al, 2003), was observed to increase the survival of B-CLL cells *in vitro*.

5.2 Use of IRES mediated translation for genes identified in microarray study

Due to the emerging importance of internal ribosome entry as a mechanism of translation regulation, and the reduction in overall translation in B-CLL cells observed in experiments reported in chapter 3, it was decided to investigate this as a potential mechanism of translation up-regulation used in B-CLL. Three genes identified in the microarray study, Bcl2, AGTR1 and eIF4G2 (DAP5), have previously been found to contain IRES elements in the 5'UTR of their mRNAs (Henis-Korenblit et al., 2000; Sherrill et al., 2004; Martin et al., 2003). In contrast, IRES elements have also been found in the 5'UTRs of eIF4GI and

ODC1 (ornithine decarboxylase 1) (Johannes and Sarnow, 1998; Pyronnet et al., 2000), which were found to have decreased polysome association in B-CLL cells. This did not give much insight into the importance of this mode of regulation in B-CLL cells and therefore further analysis was required to determine whether it could be an important mechanism of translation regulation used in B-CLL.

5.3 Evidence for the presence of IRES elements in the 5'UTRs of BTG1, UCP2 and CSNK2B

IRES activity was initially analysed for three mRNAs observed to have increased polysome association in B-CLL cells by the microarray analysis performed (section 4.2). All three messages were observed to have consistently increased polysome association for most of the B-CLL patients when compared to that for tonsil CD19+ B cells, except CSNK2B where three patients appear to have no change (Figure 5.1). The increased polysome association was also confirmed for all three mRNAs by semi-quantitative RTPCR (Figure 4.12). They were therefore assumed to have increased translation in B-CLL cells.

To test for IRES activity, the dicistronic reporter vector pRF was used (Figure 5.2). Translation of the *Renilla* luciferase is initiated in a cap-dependant manner whilst translation initiation of the firefly luciferase is cap-independent and requires an IRES upstream. Thus 5'UTRs thought to contain an IRES can be inserted between the two cistrons and, if an increase in firefly luciferase translation is observed, assumed to contain an IRES. The 5'UTRs for BTG1, UCP2 and CSNK2B were obtained by RTPCR amplification, using cDNA synthesised from B-CLL total RNA. Each 5'UTR was then digested with the restriction enzymes *Eco*RI and *Nco*I and inserted into the intercistronic spacer region of pRF between these sites (Figure 5.2). The constructs were then introduced into HeLa cells by transient transfection, in parallel with the empty vector pRF, and the activities of firefly and *Renilla* luciferases in lysates of transfected cells were measured. HeLa cells were used as generally, previously identified IRESes have good activity in these cells, probably due to a large abundance of potential *trans*acting factors. To determine whether there was any increase in firefly translation when the 5'UTRs were inserted upstream, the amount of firefly activity relative to *Renilla* activity was calculated and compared to the relative activity of firefly for pRF, where any firefly produced occurs through ribosome readthrough. The results reveal all three 5'UTRs cause an increase in firefly activity over that observed for readthrough, therefore it would be predicted that they

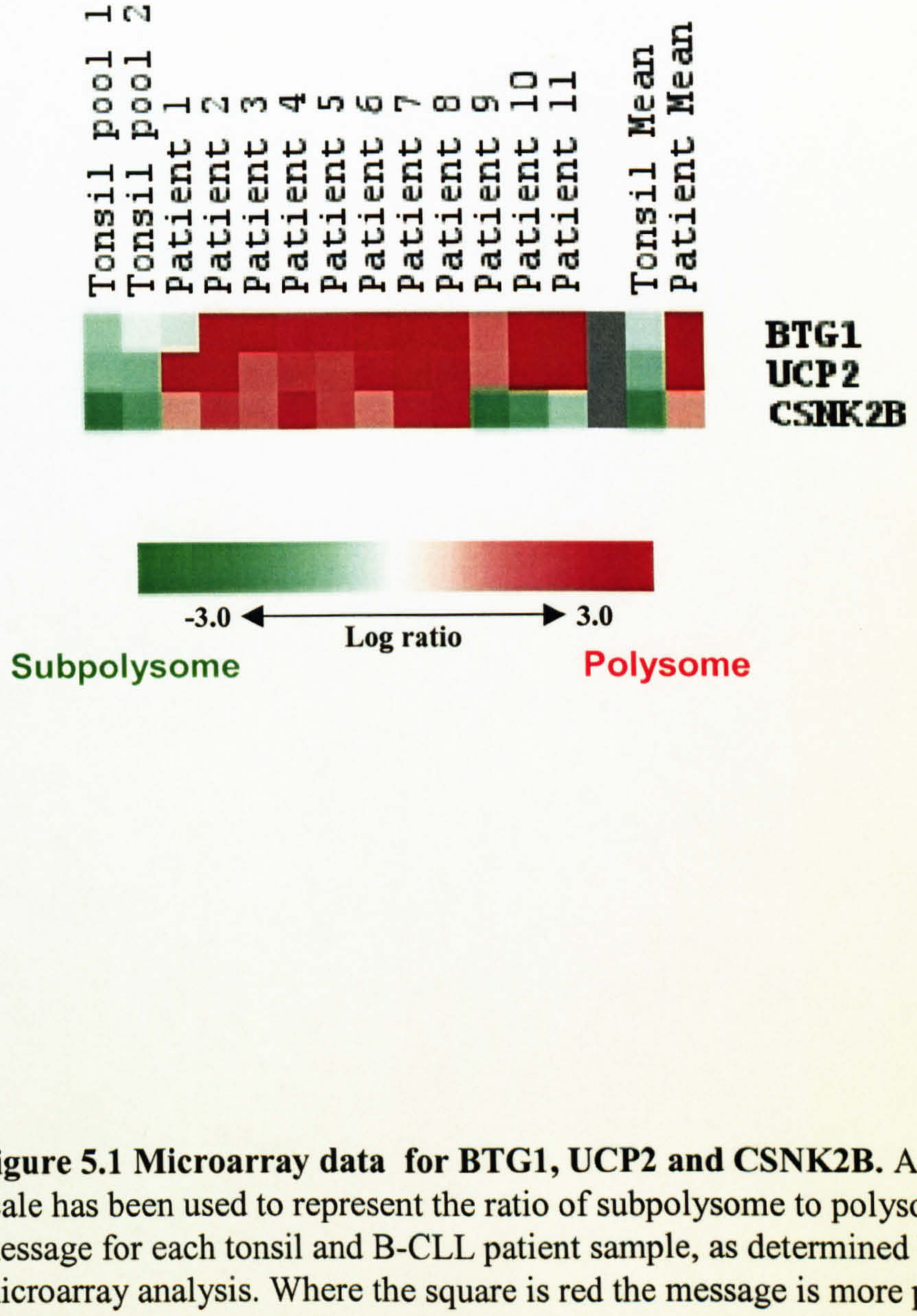
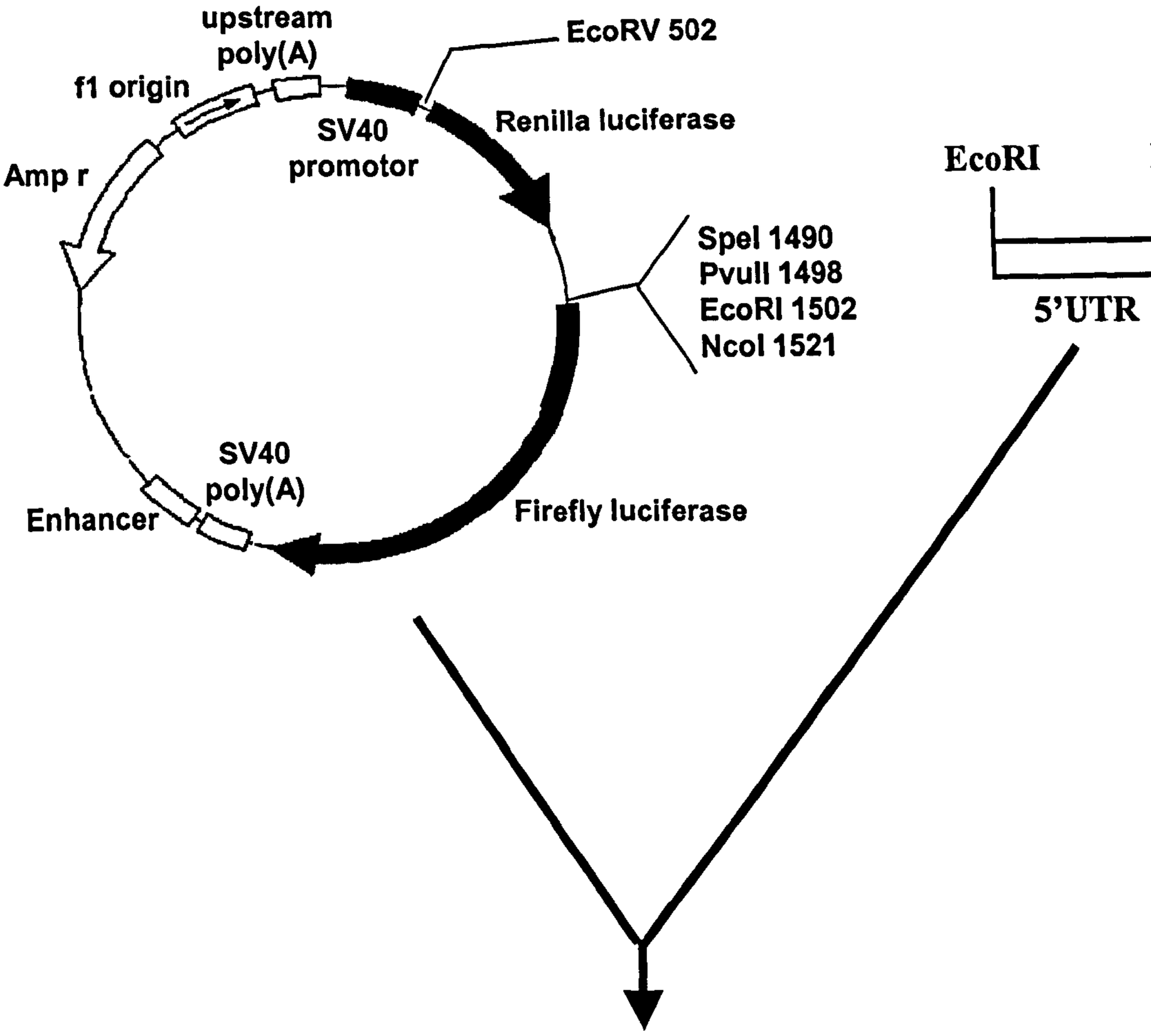


Figure 5.1 Microarray data for BTG1, UCP2 and CSNK2B. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample, as determined by cDNA microarray analysis. Where the square is red the message is more polysome associated and where green more subpolysome associated. For all three genes, an increase in polysome associated message is observed for the majority of B-CLL patients, compared to tonsil CD19+ B cells.



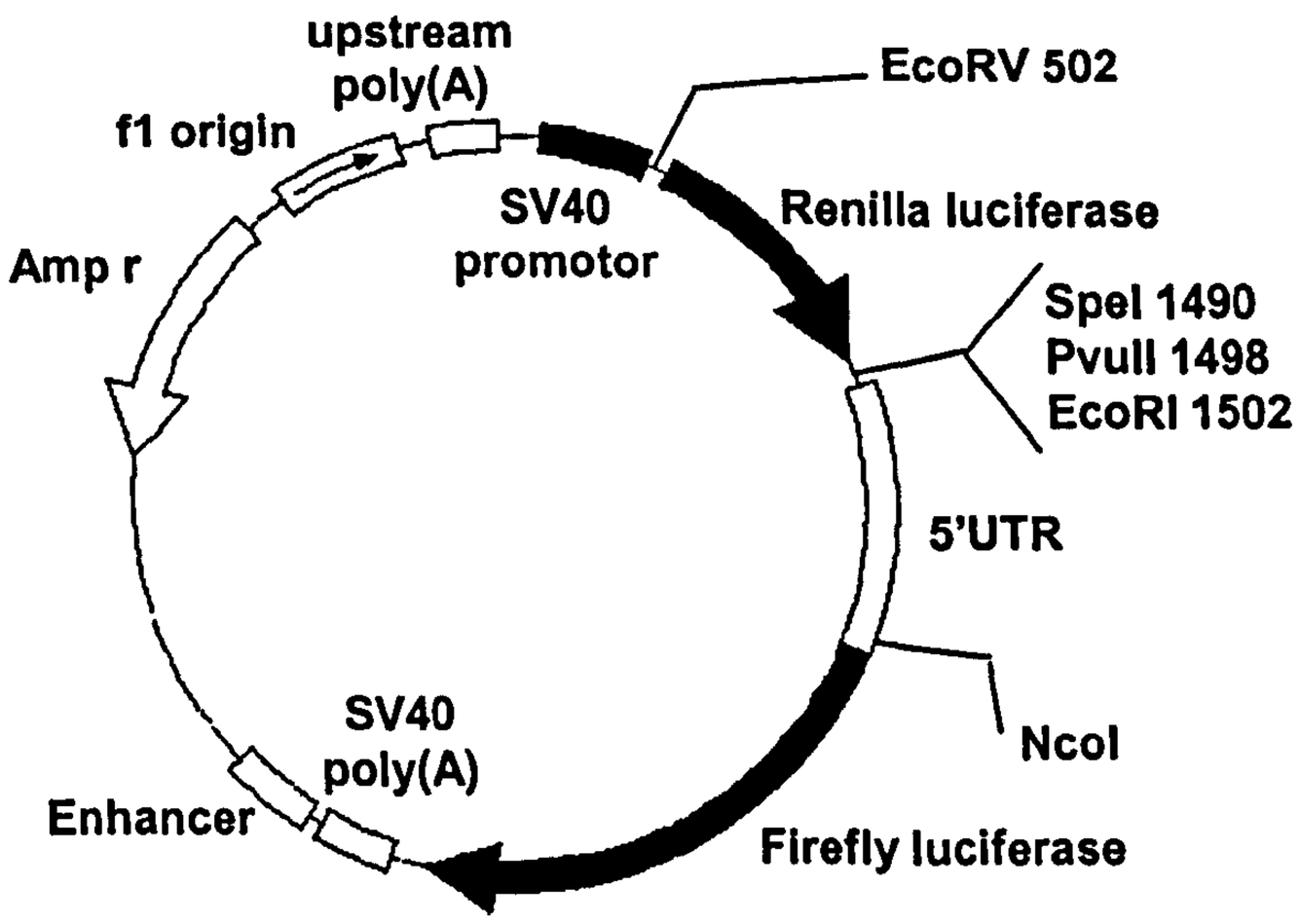


Figure 5.2 Cloning strategy to test for IRES activity. RTPCR products encoding the 5'UTRs of BTG1, UCP2 and CSNK2B were digested with *Eco*RI and *Nco*I and inserted between these sites in the vector pRF after digestion and dephosphorylation of the vector.

contain IRES elements (Figure 5.3). For UCP2 and CSNK2B, there was a 16.3-fold and 11.3-fold increase in relative firefly activity over that for pRF respectively, indicating they had reasonably active IRES elements contained in their 5'UTRs. For BTG1 there was only a 6.4-fold increase, which was not as large but enough increase to suggest that IRES activity was occurring. However, further experimentation would be required to confirm that the results observed were a consequence of IRES activity as other possible explanations, such as splicing or cryptic promoters, could also account for the data observed.

5.4 Establishment of an arrested state in CHOK1 cells

The presence of IRES elements in the mRNAs of BTG1, UCP2 and CSNK2B does not necessarily mean they are being used in B-CLL cells to increase translation of the messages. B-CLL cells cannot be cultured *in vitro* as they spontaneously apoptose (Collins et al., 1989; MacFarlane et al., 2002), so a direct measure of IRES activity in B-CLL cells cannot be obtained. B-CLL cells have been observed to be quiescent (Andreeff et al., 1980; Caligaris-Cappio and Hamblin, 1999) and experiments presented in chapter 3 suggest they have reduced rates of translation. Therefore, IRES activity was measured under conditions where cells were arrested by serum starvation. The experiments were performed using CHOK1 cells as these are not a tumour cell line so would more readily arrest. To determine whether cells were arrested when grown under conditions of serum starvation protein levels of cyclin D2 were analysed as this has been observed to be up-regulated under conditions of growth arrest, including serum starvation (Meyyappan et al., 1998). CHOK1 cells were grown for 24 hours in media containing 10% or 0.1% serum then lysed in SDS sample buffer, separated by SDS-PAGE and immunobloted. As expected Western analysis revealed there was an increase in the amount of cyclin D2 protein in cells grown with only 0.1% serum rather than 10% (Figure 5.4). In addition, only full length PARP was present in cells grown under both sets of conditions so the cells were not apoptosing (Figure 5.4). Increased levels of cyclin D2 were also observed for B-CLL cells when compared to tonsil CD19+ B cells as presented in chapter 4 (Figure 4.13). This data suggests the CHOK1 cells were induced to arrest when grown under serum starvation conditions.

5.5 Global rates of translation were reduced under serum starvation conditions

The rate of translation was measured for CHOK1 cells grown under normal and serum starvation conditions. After 24 hours growth in media containing 10% or 0.1% serum, 10μCi ^{35}S methionine was added to a 10cm^2 plate. Cells were then grown for a further 20

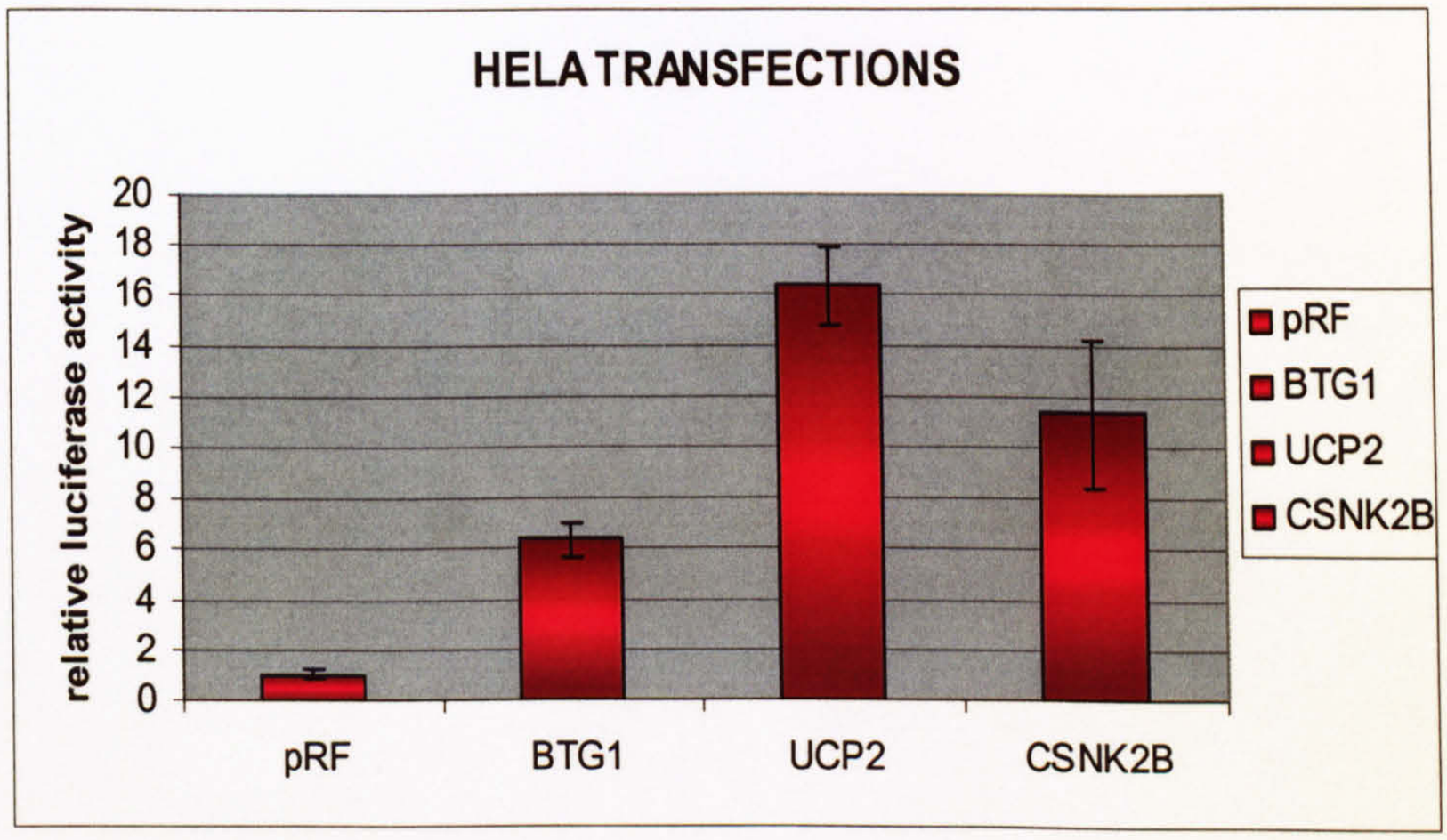


Figure 5.3 Determination of IRES activity in HeLa cells. Dicistonic pRF constucts containing the 5'UTRs for BTG1, UCP2 and CSNK2B were transfected into HeLa cells and the amount of luciferase protein produced assayed. Where an IRES is present the amount of Firefly activity relative to Renilla is increased compared to that recorded for the empty construct. This was observed for all three 5'UTRs tested. The experiment was performed once in triplicate.

Figure 5.4 Determination of growth status in CHOK1 cells using Western blot analysis. Cell lysates were made from CHOK1 cells subjected to serum starvation (0.1% serum) or control (10% serum) conditions for 24 hours and separated by SDS PAGE. The gels were then immunoblotted and probed with specific antibodies to cyclin D2, PARP and actin. An increase in cyclin D2 levels were observed in serum starved cells, indicating that they were arrested. Plus no PARP cleavage was seen suggesting the cells were not apoptosing. Each Western was performed once.

minutes before being harvested and incorporation of ^{35}S methionine into proteins determined. A 70% reduction in the amount of translation occurring was observed in the serum starved cells (Figure 5.5). Therefore global rates of translation were reduced under conditions of serum starvation induced growth arrest. The data presented in chapter 3 suggests that a reduced level of translation may be occurring in B-CLL cells, plus an increased level of cyclin D2 protein was observed (Figure 4.13), therefore IRES activity analysed under serum starvation conditions may reflect what occurs in B-CLL cells.

5.6 IRES activity for BTG1, UCP2 and CSNK2B was maintained under conditions of serum starvation

After establishment of serum starvation conditions in CHOK1 cells, IRES activity for BTG1, UCP2 and CSNK2B under these conditions was determined. pRF and the constructs containing the 5'UTRs of BTG1, UCP2 and CSNK2B were introduced into CHOK1 cells by transient transfection. 24 hours after transfection the media was removed, the cells washed with PBS and new media added containing 10% or 0.1% serum. The cells were then grown for a further 24 hours before being harvested and luciferase activity assayed. IRES activity under normal growth conditions for the three 5'UTRs was not the same in CHOK1 cells as it was in HeLa cells. A reduced level of relative firefly activity was observed for all three 5'UTRs compared to that observed in HeLa cells (Figure 5.6). The relative firefly activity for UCP2 and CSNK2B 5'UTRs was 3.7 and 5.3 fold over readthrough respectively in CHOK1 cells, compared to 16.3 and 11.4 in HeLas. The reduction for BTG1 IRES activity was not as large, only from 6.4 in Hela cells to 4.7 in CHOK1 cells, which was probably due to its activity being quite low in HeLa cells. The reduction was probably due to the differences in proteins found in these cells compared to HeLa cells that act to enhance IRES mediated translation. Under serum starvation conditions all three IRESes show maintenance of activity (Figure 5.6). BTG1 and CSNK2B IRESes have slightly increased activity relative to readthrough in the serum starved cells compared to under normal growth conditions. For BTG1 the relative luciferase activity increased from 4.7 to 5.6, and for CSNK2B 5.3 to 9.1. The IRES activity for UCP2 did not increase but remained constant. This provides evidence that the same IRES activity may be found in B-CLL cells and that IRES mediated translation may be a mechanism used for up-regulation of genes.

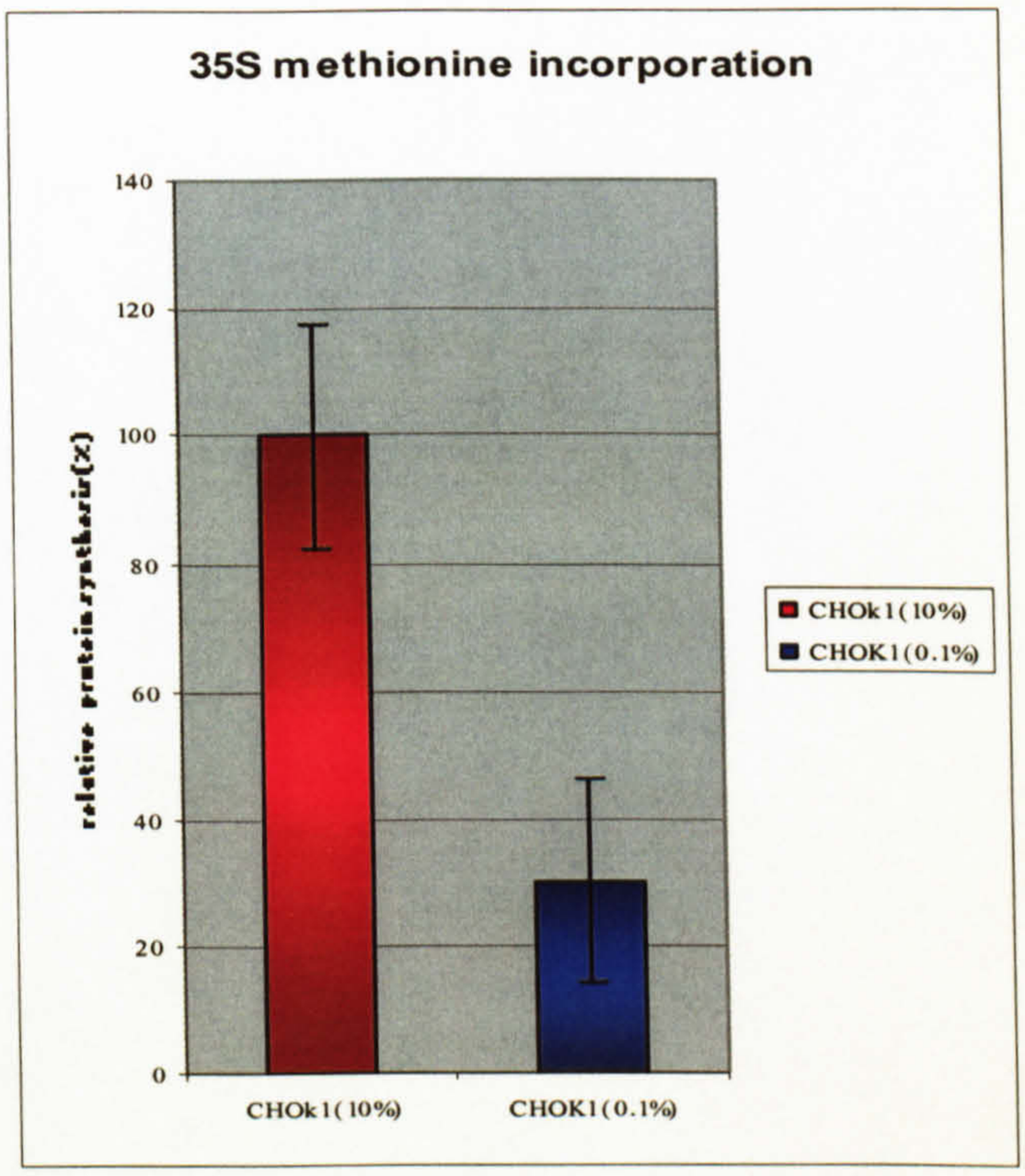


Figure 5.5 Determination of the rate of protein synthesis in CHOK1 cells by ^{35}S methionine incorporation. CHOK1 cells subjected to serum starvation (0.1% serum) or control (10% serum) conditions for 24 hours, were pulse labelled with ^{35}S methionine. The amount of incorporation was then measured and relative protein synthesis rates calculated. A 70% reduction in the level of protein synthesis is observed under serum starvation conditions. The experiment was performed once in triplicate.

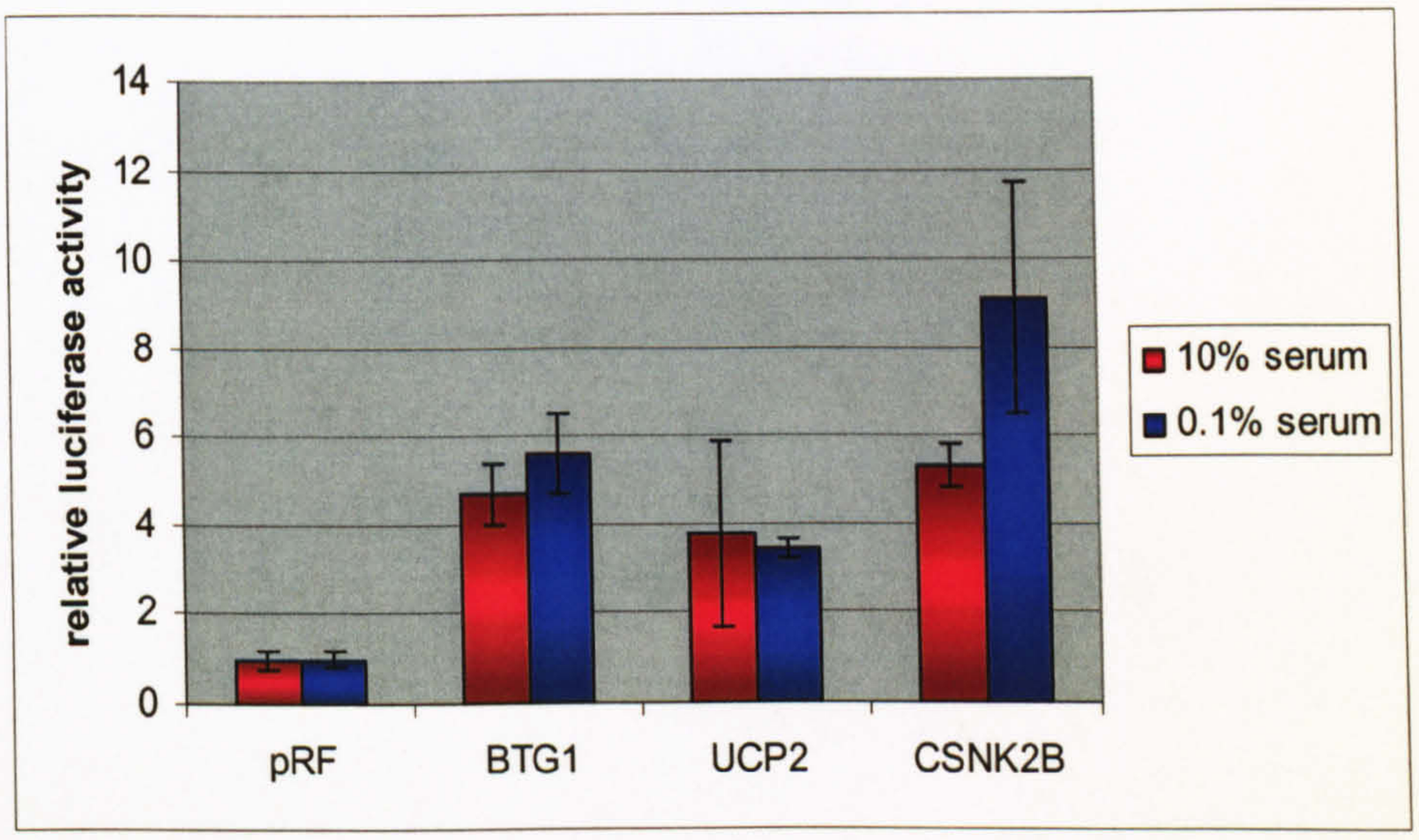


Figure 5.6 Determination of IRES activity in serum starved cells.
Dicistronic pRF constructs containing the 5'UTRs for BTG1, UCP2 and CSNK2B were transfected into CHOK1 cells. 24 hours post transfection the cells were subjected to either serum starvation conditions (0.1% serum) or remained under control conditions (10% serum), for a further 24 hours . The amount of luciferase protein produced was then assayed. The IRES activity for all three 5'UTRs was found to be increased or maintained under serum starvation conditions. The experiment was performed once in triplicate.

5.7 AGTR1 shows increased polysome association and increased protein levels in B-CLL cells

B-CLL cells are resistant to apoptosis *in vivo* yet rapidly die when cultured *in vitro* (Collins et al., 1989; MacFarlane et al., 2002). Therefore it has been suggested that survival signals from the *in vivo* microenvironment may be required for survival. One possibility may be a receptor involved in survival signalling could be up-regulated in B-CLL cells. AGTR1 (angiotensin II type 1 receptor) was identified by the microarray analysis as the receptor with the most significantly increased polysome association in B-CLL cells. Increased polysome association is consistently observed for all the B-CLL patients when compared to tonsil CD19+ B cells (Figure 5.7). There is accumulating evidence of a role for angiotensin II (the ligand for AGTR1) in cell growth, differentiation and death, as well as its well characterised role in regulating blood pressure (Dimmeler et al., 1997; Schelman et al., 2004), which suggests it could have an effect on B-CLL cell survival *in vivo*. (A more extensive description of this receptor's function is given in section 4.3). Western analysis revealed that AGTR1 protein levels were higher in B-CLL cells compared to in tonsil CD19+ B cells for most patients (Figure 5.8), therefore the increased polysome association observed was also mirrored at the protein level so may be of functional consequence. A previous study has identified an IRES element in the 5'UTR of AGTR1 mRNA which was shown to be utilised under serum starvation conditions to maintain translation (Martin et al., 2003), thus identifies IRES mediated translation as a potential mechanism used for up-regulation in B-CLL.

5.8 Angiotensin II causes an increase in survival of B-CLL cells *in vitro*

The results reveal consistent up-regulation of AGTR1 at the translation and protein level. Moreover, there is evidence that signalling through the receptor may have a role in survival. Therefore the effect of its ligand, ang II, on the survival of B-CLL cells *in vitro* was investigated. To analyse B-CLL cell survival *in vitro,* cells were isolated from patient blood samples then cultured and grown for 24 hours *in vitro*. The amount of apoptosis that had occurred was then determined by staining cells with annexin V and FACs analysing. When this was performed for B-CLL cells (patient 18) about 25% spontaneous apoptosis was observed (Figure 5.9). To determine the effects of ang II on B-CLL survival, it was added to cultures in a range of concentrations then apoptosis rates measured. Addition of ang II to B-CLL cultures caused a modest reduction in spontaneous apoptosis to about 20% (Figure 5.10) and this did not change with addition of increased concentrations, therefore

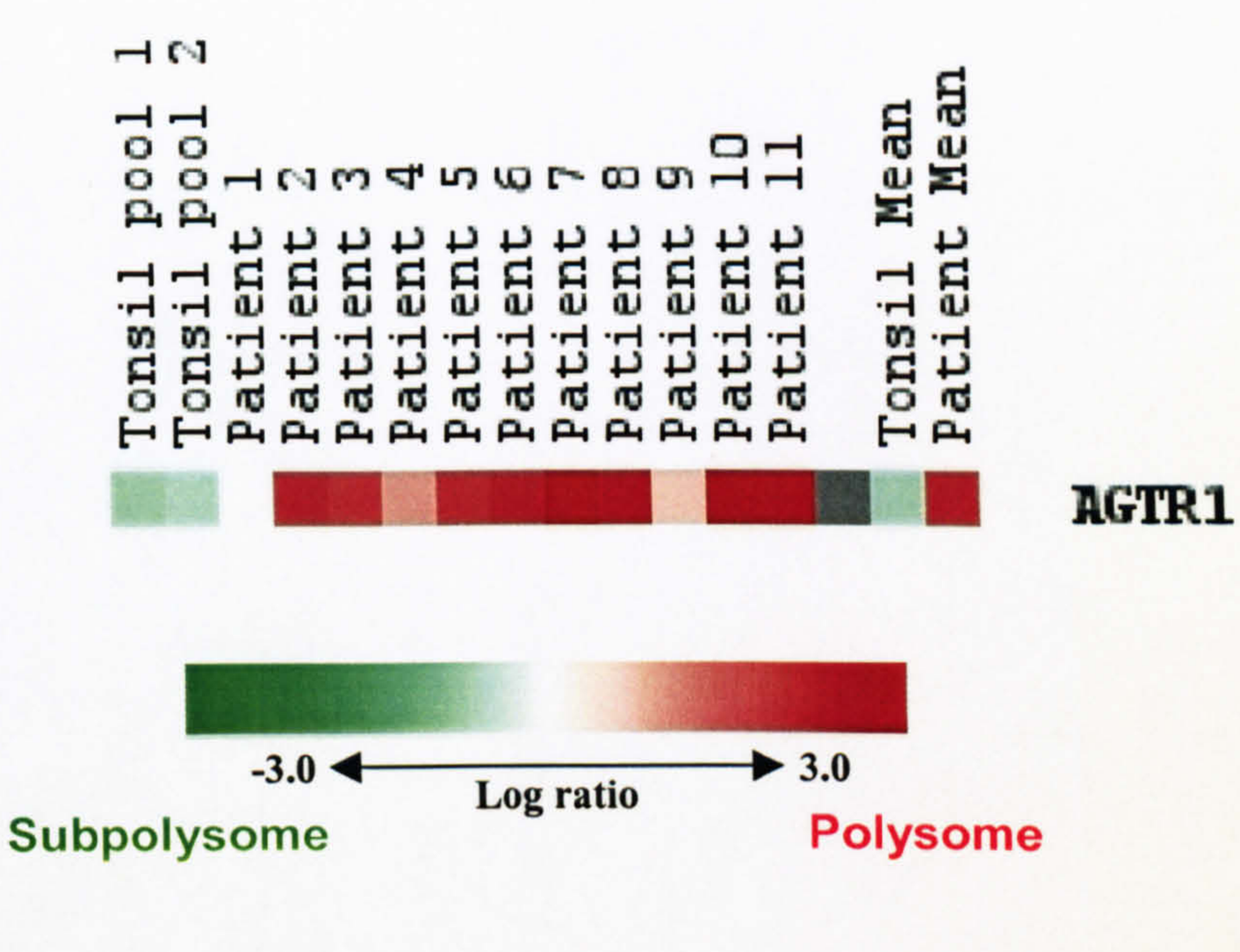


Figure 5.7 Microarray data for AGTR1. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample, as determined by cDNA microarray analysis. Where the square is red the message is more polysome associated and where green more subpolysome associated. an increase in polysome associated message is observed for all of the B-CLL patients, compared to tonsil CD19+ B cells.

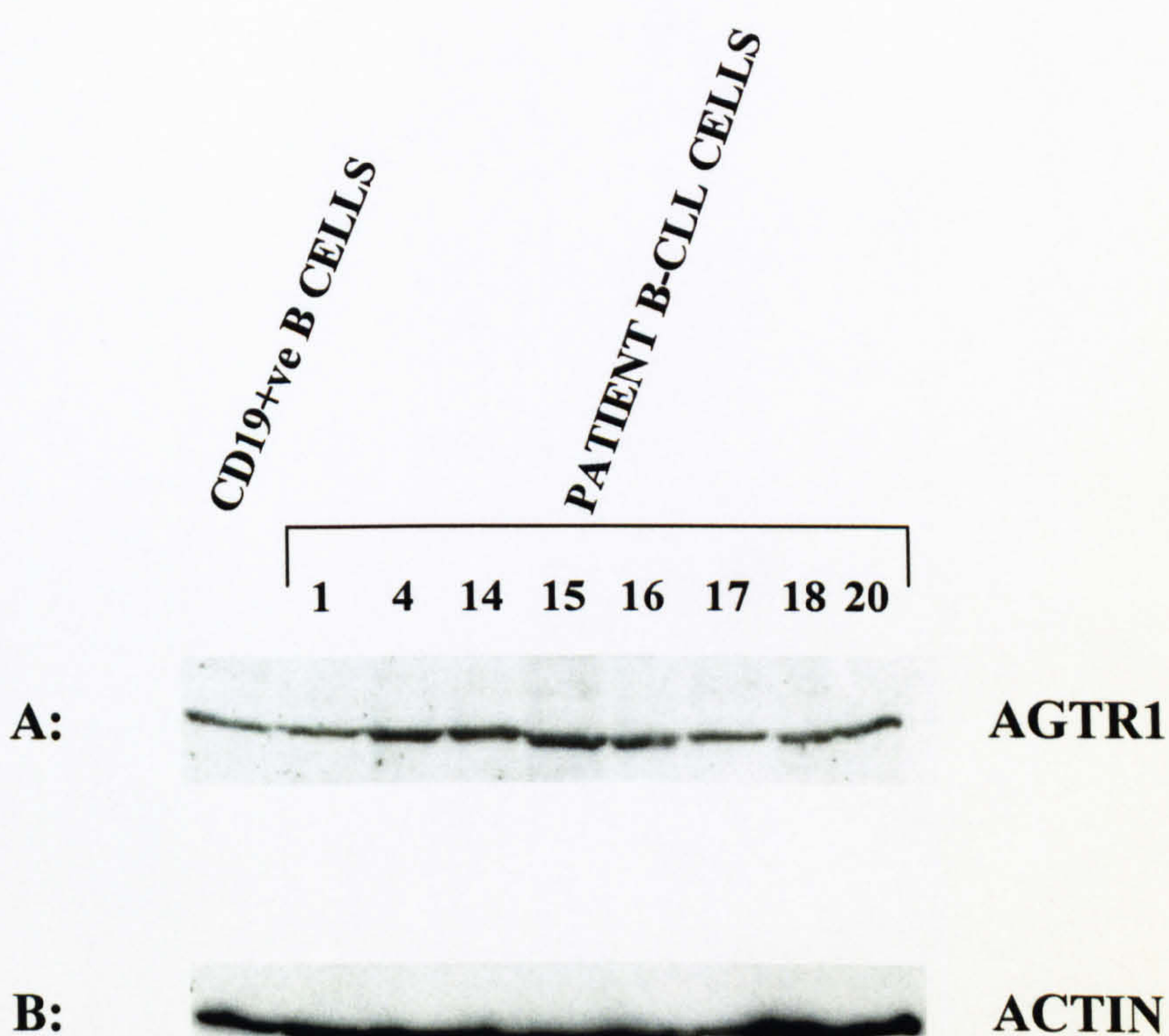
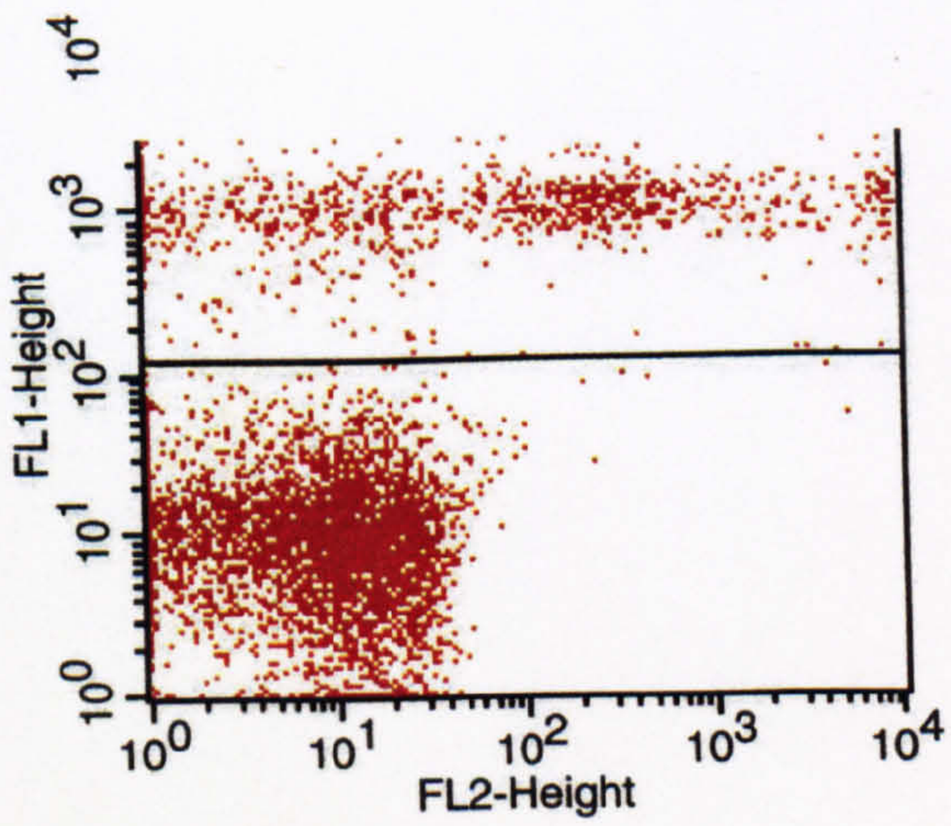


Figure 5.8 Western analysis of AGTR1 protein levels in B-CLL cells. Cell lysates were made from B-CLL cells (patients 1, 4, 14, 15, 16, 17, 18 and 20) and tonsil CD19+ B cells and separated by SDS PAGE. The gels were then immunoblotted and probed with specific antibodies to AGTR1 and actin. A higher level of AGTR1 protein was observed for most B-CLL patients compared to tonsil CD19+ B cells. Each Western was performed once.



ANNEXINV+ 25.04%

ANNEXINV- 74.96%

Figure 5.9 FACS analysis of spontaneous apoptosis in B-CLL cells.
Purified B-CLL cells (patient 18) were cultured for 24 hours in RPMI media + 10% serum. After incubation at 37°C for 24 hours the percentage of spontaneous apoptosis that had occurred was measured by staining the cells with annexinV and FACS. 25.04% spontaneous apoptosis had occurred, which is a typical percentage observed for B-CLL patient samples. The experiment was performed once.

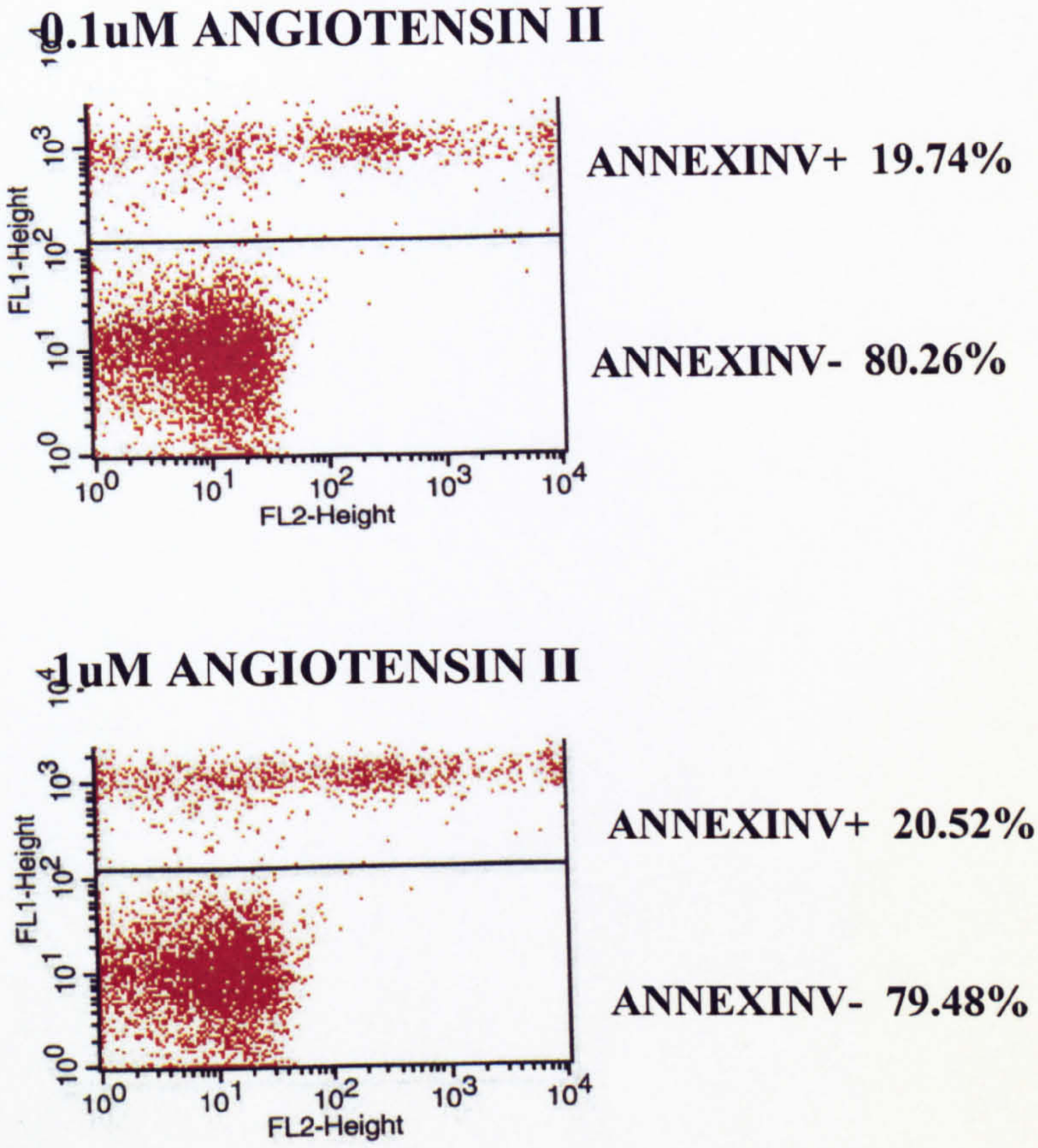


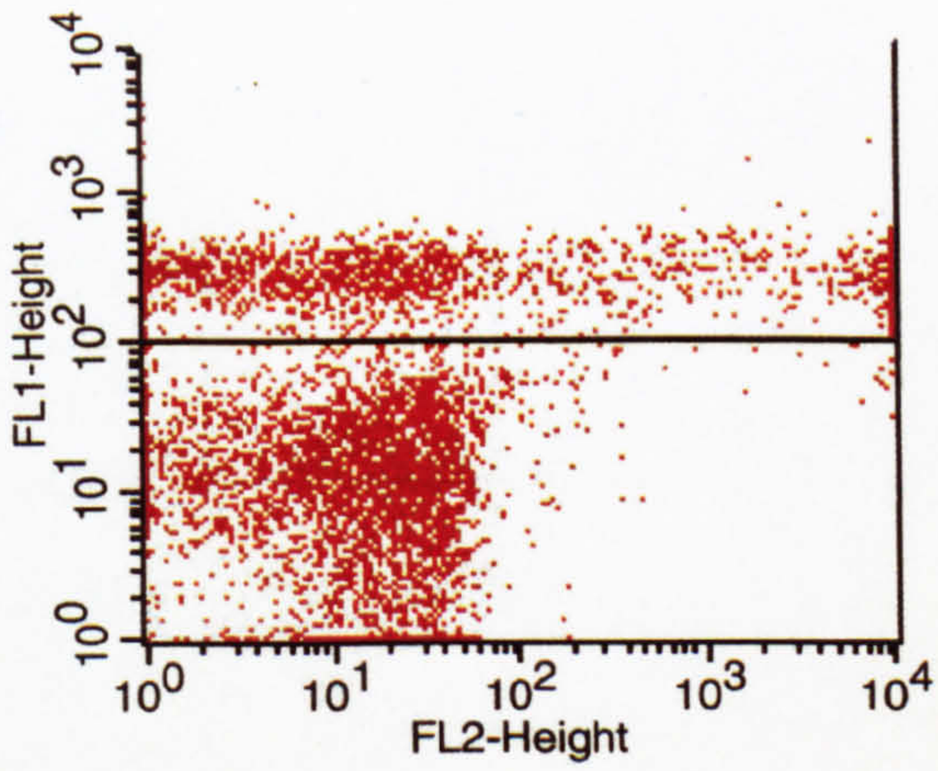
Figure 5.10 Affect of angiotensin II on B-CLL cell survival. Purified B-CLL cells (patient 18) were cultured for 24 hours in RPMI media + 10% serum + angiotensin II at a concentration of 0.1μM, 1μM or 100μM. After incubation at 37°C for 24 hours the percentage of spontaneous apoptosis that had occurred was measured by staining the cells with annexinV and FACS. A moderate reduction in apoptosis was observed, suggesting angiotensin II may play a role in survival of B-CLL cells. The experiment was performed once.

ang II may not be having much effect on B-CLL cell survival. However the experiment was repeated for a further three patients (numbers 21, 22 and 23), resulting in an average reduction in apoptosis of 7.1% being observed (data not shown). The consistant reduction observed therefore suggests ang II was having some effect. It is suggested that ang II may be present in the serum used in the growth medium thus may be saturating receptors and reducing any survival effect observed.

To determine whether the effects on apoptosis observed were specific for B-CLL cells, the experiment was also performed with tonsil CD19+ B cells. When cultured spontaneous apoptosis also occurred in these cells; after 24 hours about 30% apoptosis was observed (Figure 5.11). However, there was no change in apoptosis rates when ang II was added (Figure 5.12), which suggests that the increase in survival observed in B-CLL cells was specific and may be as a result of the increased protein levels of AGTR1. (All FACs analysis was performed by Dr Marion MacFarlane).

5.9 Discussion

To attempt to gain some insight into the mechanism(s) of translation regulation utilised by genes identified in the microarray analysis presented in chapter 4, some initial experiments were performed to determine if IRES mediated translation may be used as a mechanism of upregulation. Only five genes had previously been found to use IRES mediated translation therefore further analysis was required to determine if this could be a widely utilised mechanism in B-CLL cells. Three genes were chosen for initial analysis; BTG1, UCP2 and CSNK2B, which were all observed to have consistently increased polysome association in B-CLL cells when compared to tonsil CD19+ B cells by microarray analysis and was confirmed by semi-quantitative RTPCR. The 5'UTR for each mRNA was cloned into the discistronic reporter vector and the constructs transiently transfected into HeLa cells to measure IRES activity. All three were observed to have IRES activity, although the higher activities recorded for UCP2 and CSNK2B 5'UTRs suggests they work more efficiently than that for BTG1. IRES activity cannot be measured directly in B-CLL cells as they cannot be cultured *in vitro*. B-CLL cells are quiescent therefore it was decided to test IRES activity under conditions of growth arrest. CHOK1 cells were used and were arrested under conditions of serum starvation. This caused an increase in cyclin D2 protein levels and a decrease in global translation rates, which are conditions observed in B-CLL cells. IRES activity was observed to be maintained under these conditions, for all three genes. This



ANNEXINV+ 32.71%

ANNEXINV- 67.29%

Figure 5.11 FACS analysis of spontaneous apoptosis in tonsil CD19+ B cells. Purified tonsil CD19+ B cells were cultured for 24 hours in RPMI media + 10% serum. After incubation at 37°C for 24 hours the percentage of spontaneous apoptosis that had occurred was measured by staining the cells with annexinV and FACS. The experiment was performed once.

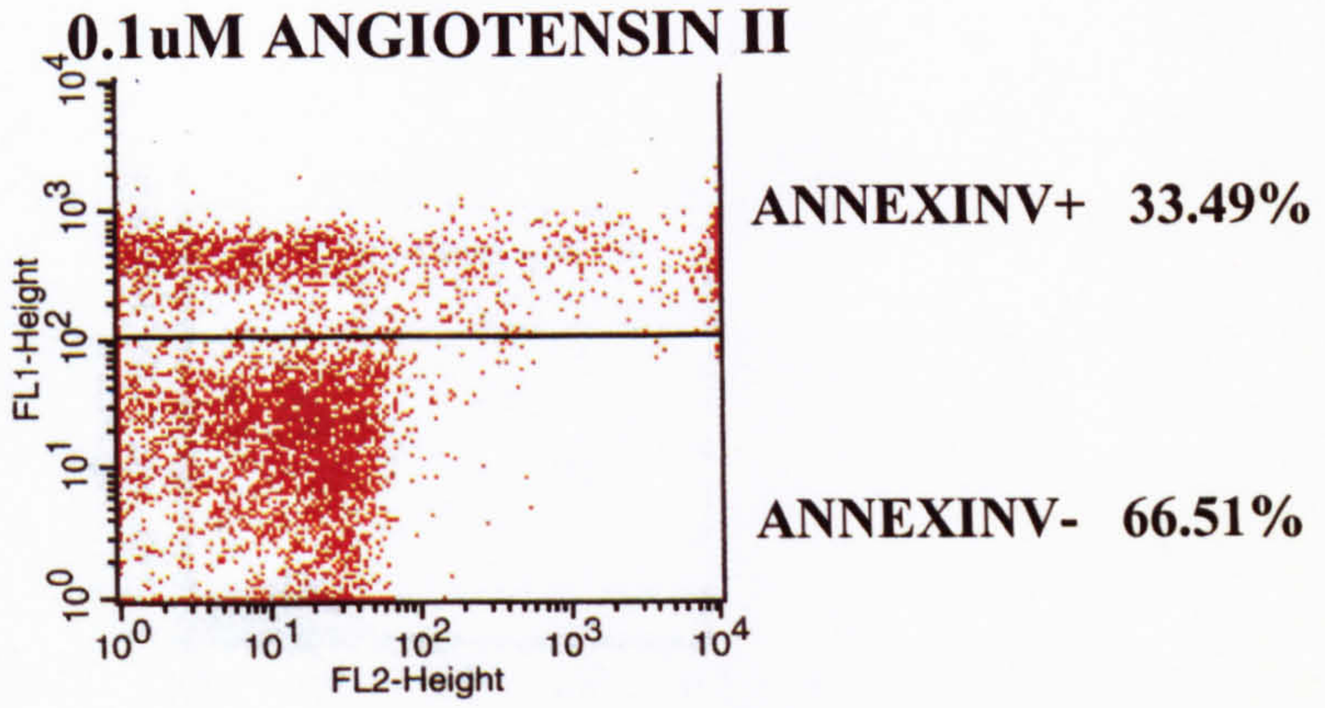


Figure 5.12 Affect of angiotensin II on tonsil CD19+ cell survival.
Purified tonsil CD19+ B cells were cultured for 24 hours in RPMI media + 10% serum + angiotensin II at a concentration of 0.1μM, 1μM or 100μM. After incubation at 37°C for 24 hours the percentage of spontaneous apoptosis that had occurred was measured by staining the cells with annexinV and FACS. No change in the amount of spontaneous apoptosis occurring was observed when angiotensin II was added which suggests the effect on B-CLL cells was specific. The experiment was performed once.

result provides evidence that IRES mediated translation may be utilised as a mechanism of up-regulation in B-CLL cells.

Any of the genes found with differential expression in B-CLL cells may have a significant effect on the biology of the disease. In particular, there may be changes which affect the survival of B-CLL cells *in vivo*. The effect of the ligand for the receptor AGTR1, angiotensin II on the survival of B-CLL cells was analysed. AGTR1 showed a consistent increase in polysome associated mRNA for all B-CLL patients compared to tonsil CD19+ B cells and was also observed to be increased at the protein level. B-CLL cells were isolated and cultured *in vitro* and ang II added to determine if it affected the amount of spontaneous apoptosis. Ang II was observed to reduce the amount of apoptosis that occurred on average by about 7%. This effect was shown to be specific for B-CLL cells as no effect was seen in tonsil CD19+ B cells. The reduction in apoptosis was only moderate therefore ang II may not have a significant effect on B-CLL cell survival. However, it is suggested that ang II may be present in the serum used in the growth medium thus may be saturating receptors and reducing any survival effect observed. Further analysis would be required to determine if this was the case. However initial evidence suggests that ang II might have an effect on B-CLL cell survival *in vivo* acting through the AGTR1 receptor and makes this receptor a potential new target for treatments. Drugs have previously been developed that specifically target the AGTR1 receptor for use in treating hypertension therefore are already available if the effect is proven to be real.

Chapter 6

cDNA microarray analysis of differential translation depending upon I_gV_H mutational status

6.1 Introduction

Microarray analysis has been used previously to determine gene expression differences at the level of transcription between the B-CLL subtypes defined by I_gV_H gene mutation status (Klein et al., 2001; Rosenwald et al., 2001). These studies determined that both subtypes shared a common expression profile so were the same disease, but also identified differences that could potentially be utilised as prognostic markers. However, there may also be differences in gene expression at the level of translation that may be of importance. Therefore, a comparison of the polysome association of mRNAs for patients in each subgroup was analysed using the microarray obtained from experiments presented in chapter 4 and differences identified.

6.2 Analysis of ZAP70 protein levels in B-CLL patients

As mentioned previously, ZAP70 was one of the genes observed by microarray analysis to be differentially expressed in B-CLL depending on I_gV_H gene mutation (Klein et al., 2001; Rosenwald et al., 2001), and in addition was found to show the same pattern of expression at the protein level (Crespo et al., 2003) so could therefore be used as a prognostic marker. ZAP70 levels were analysed for the B-CLL patients used in the microarray study (Figure 6.1) and observed to be reduced in two of the patients (7 and 10). Low ZAP70 levels correlate with mutated I_gV_H genes and the nonaggressive form of B-CLL and high levels with unmutated I_gV_H and the aggressive form. The proportion of patients who have the aggressive form is about 30% whereas in the patient pool used in this study it appears to be about 80%. The difference was probably reflects the size of the patient pool used.

6.3 Microarray analysis of translation comparing patients depending upon their ZAP70 level

The microarray data for the patients with each subtype was compared to identify any differences in gene expression at the level of translation between the two. 239 genes were identified with differential polysome association. 125 genes had higher polysome association in patients with low ZAP70 levels (Figure 6.2 and Appendix 8) and 114 were

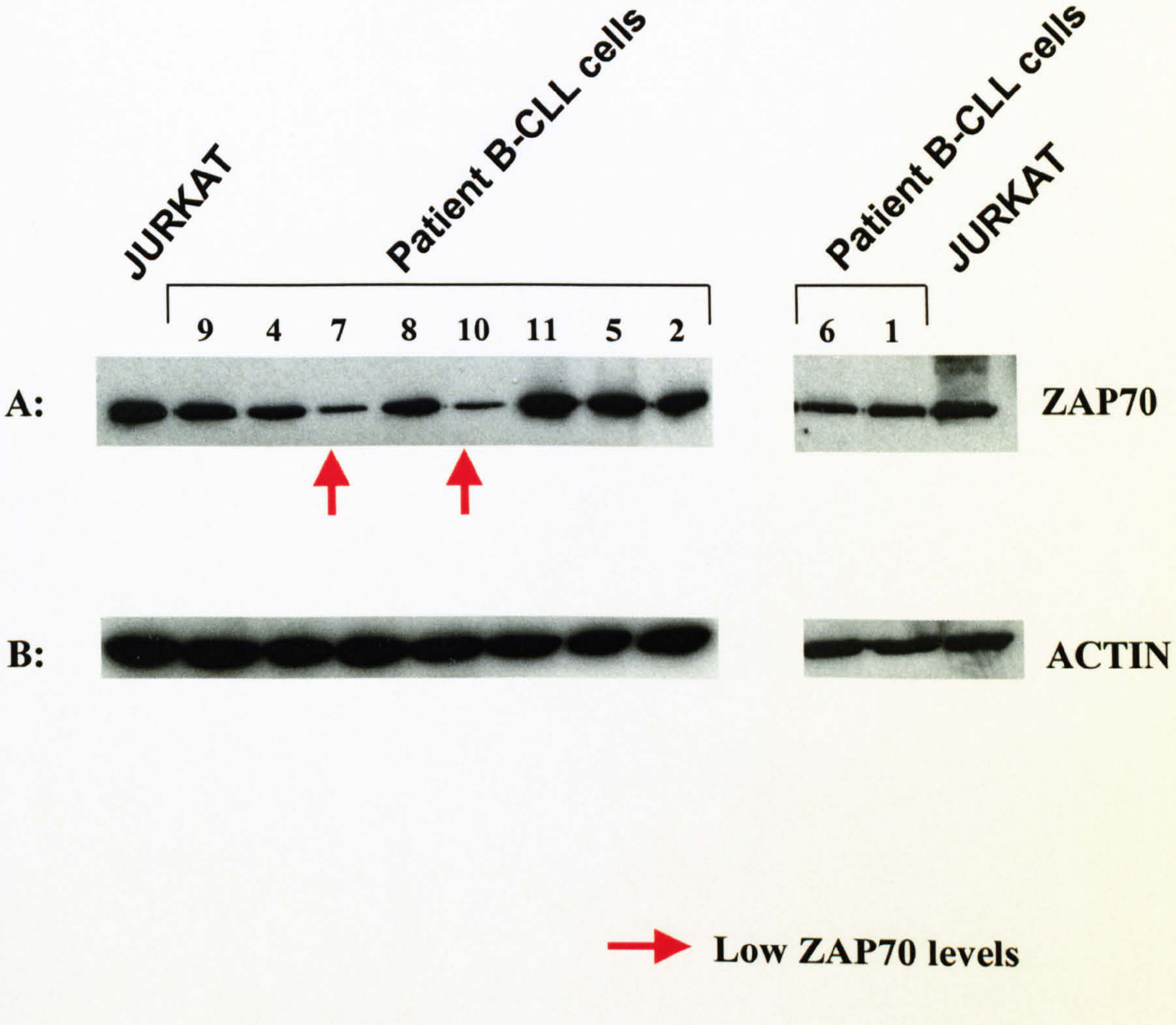
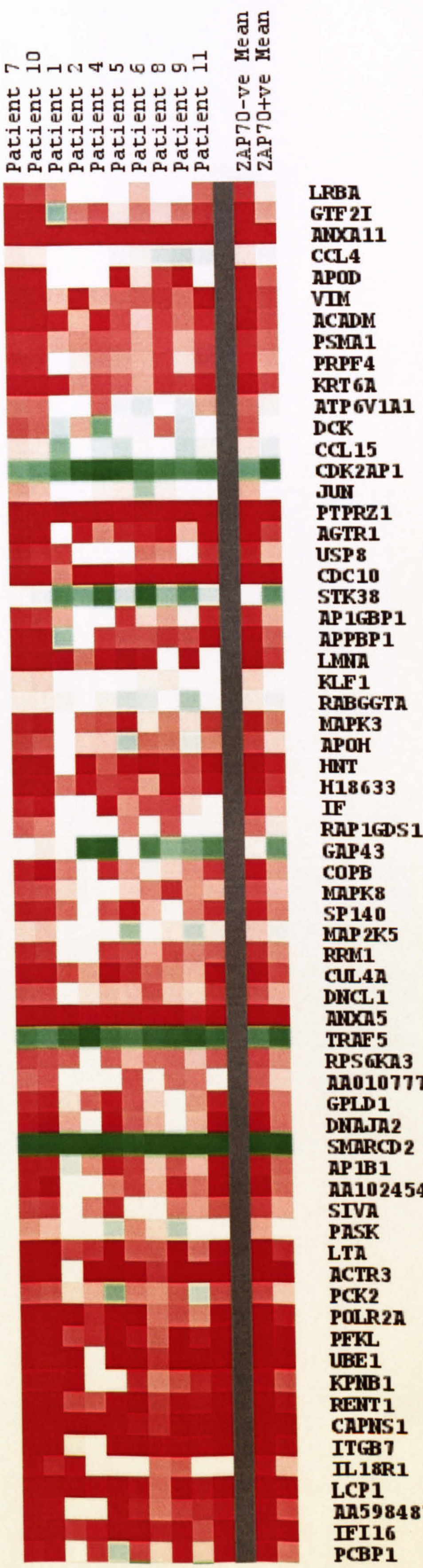


Figure 6.1 Western analysis of ZAP70 expression in B-CLL patients. Cell lysates were made from B-CLL cells (patients 1-11, excluding 3) and Jurkat cells and separated by SDS PAGE. The gels were then immunoblotted and probed with specific antibodies to ZAP70 (A) and actin (B). The red arrows highlight the patients that had low ZAP70 protein levels, which were patients 7 and 10. Each Western was performed once.


Patient 7
Patient 10
Patient 1
Patient 2
Patient 4
Patient 5
Patient 6
Patient 8
Patient 9
Patient 11
ZAP70-ve Mean
ZAP70+ve Mean
LRBA
GTF2I
ANXA11
CCL4
APOD
VIM
ACADM
PSMA1
PRPF4
KRT6A
ATP6V1A1
DCK
CCL15
CDK2AP1
JUN
PTPRZ1
AGTR1
USP8
CDC10
STK38
AP1GBP1
APPBP1
LMNA
KLF1
RABGGTA
MAPK3
APOH
HNT
H18633
IF
RAP1GDS1
GAP43
COPB
MAPK8
SP140
MAP2K5
RRM1
CUL4A
DNCL1
ANXA5
TRAF5
RPS6KA3
AA010777
GPLD1
DNAJA2
SMARCD2
AP1B1
AA102454
SIVA
PASK
LTA
ACTR3
PCK2
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RENT1
CAPNS1
ITGB7
IL18R1
LCP1
AA598487
IFI16
PCBP1

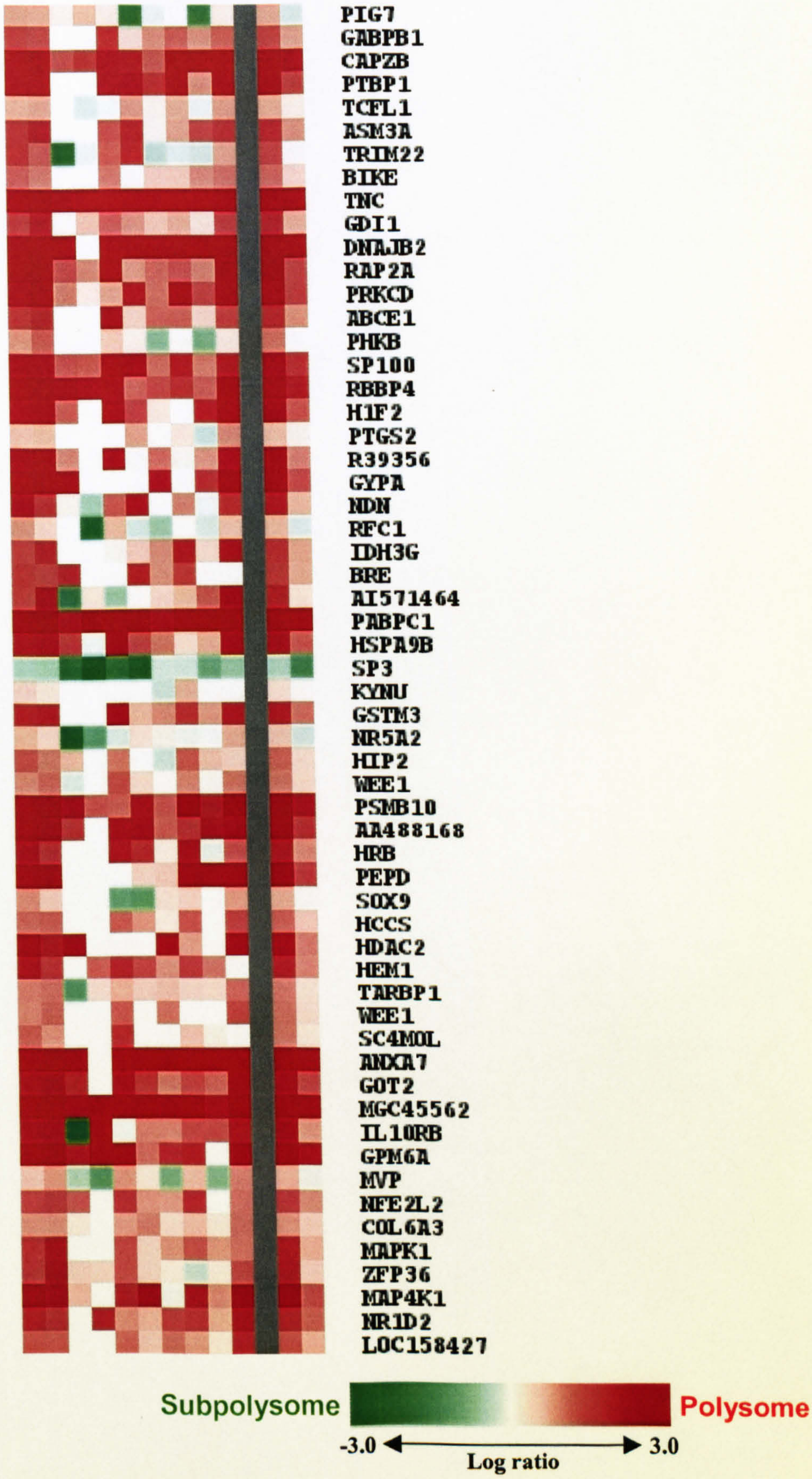


Figure 6.2 Genes with higher polysome association in patients with low ZAP70- levels compared to patients with high ZAP70. A colour scale has been used to represent the ratio of subpolysome to polysome message for each B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated. Patients 7 and 10 had low ZAP70 levels and the rest high.

higher in patients with high ZAP70 levels (Figure 6.3 and appendix 9). The data may not be relevant to ZAP70 status however due to the low number of patients used in the analysis, so differences may be due to individual patient variation. Western analysis was performed using protein samples from a different set of patients from those used for the microarray study (patients 14, 16, 17, 19 and 20), plus two used in the microarray analysis (patients 1 and 4). ZAP70 protein levels, as well as two genes identified as correlating with ZAP70 status from the microarray analysis, RIPK1 and AGTR1 were determined (Figure 6.4). AGTR1 was observed to have higher polysome association in patients with low ZAP70 levels (Figure 6.2), whilst RIPK1 had lower polysome association (Figure 6.3). For this set of B-CLL patients RIPK1 levels do correlate with ZAP70 status; low ZAP70 and RIPK1 levels were observed for patients 14 and 17 suggesting, for RIPK1, protein levels may correlate with disease subtype. However, AGTR1 levels do not correlate, which suggests the variation observed is due to individual patient variation or could correlate with another disease marker such as CD38 expression. It also reflects the inaccuracies produced by the low patient numbers used.

A small group of genes show a very distinct difference in polysome association between the two subtypes, which suggests that they may correlate with ZAP70 levels. These are IGLL1 (immunoglobulin lamda like polypeptide 1), IGLJ3 (Immunoglobulin lamda joining 3), INHBA (inhibin, beta A), MTR (5-methyltetrahydrofolate-homocysteine methlytransferase), CASP1 (caspase 1) and TFAM (mitochondrial transcription factor A). They all have reduced polysome association in the ZAP70 negative patients (Figure 6.3). The polysome association observed for this group of genes for patient 11 correlated with the ZAP70 low patients, despite a high ZAP70 level being observed. ZAP70 protein levels do not correlate 100% with the I_gV_H gene mutation status, therefore this patient might have been placed in the wrong group and could actually have mutated I_gV_H genes.

IGLL1 (immunoglobulin lamda like polypeptide 1) and IGLJ3 (immunoglobulin lamda joining 3) are both components for the B cell antigen receptor (BCR). The BCR is expressed in the plasma cell membrane and is composed of disulphide bonded heavy and light immunoglobulin chains associated with a CD79α/CD79ß heterodimer. B cells develop clonally restricted antigen specific receptors. Development of B cells proceeds through a number of stages. ProB cells are the first B lineage compartment and where heavy chain

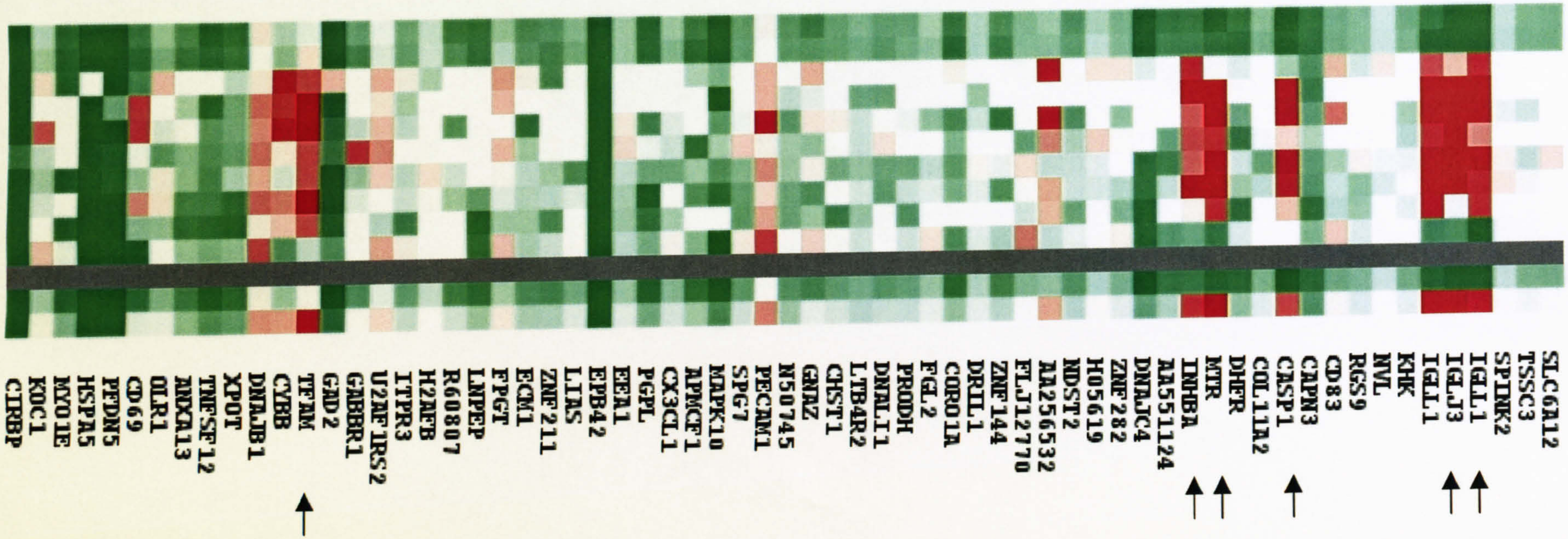

Patient 7
Patient 10
Patient 1
Patient 2
Patient 4
Patient 5
Patient 6
Patient 8
Patient 9
Patient 11
ZAP70-ve Mean
ZAP70+ve Mean
SLC6A12
TSSC3
SPINK2
IGLL1
IGLJ3
IGLL1
KHK
NVL
RGS9
CD83
CAPN3
CASP1
COL11A2
DHFR
MTR
IMMBA
AA551124
DNAJC4
ZNF282
H05619
NDST2
AA256532
FLJ12770
ZNF144
DRIL1
CORO1A
FGL2
PRODH
DNALI1
LTB4R2
CHST1
GNAZ
N50745
PECAM1
SPG7
MAPK10
APMCF1
CX3CL1
PGPL
EEA1
EPB42
LIAS
ZNF211
ECM1
FPGT
LNPEP
R60807
H2AFB
ITPR3
U2AF1RS2
GABBR1
GAD2
TFAM
CYBB
DNAJB1
XPOT
TNFSF12
ANXA13
OLR1
CD69
PFDN5
HSPA5
MYO1E
KOC1
CIRBP

Figure 6.3 Genes with higher polysome association in patients with high ZAP70 levels compared to patients with low ZAP70. A colour scale has been used to represent the ratio of subpolysome to polysome message for each B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated. Patients 7 and 10 had low ZAP70 levels and the rest high. Those genes which are of particular interest are highlighted by arrows.

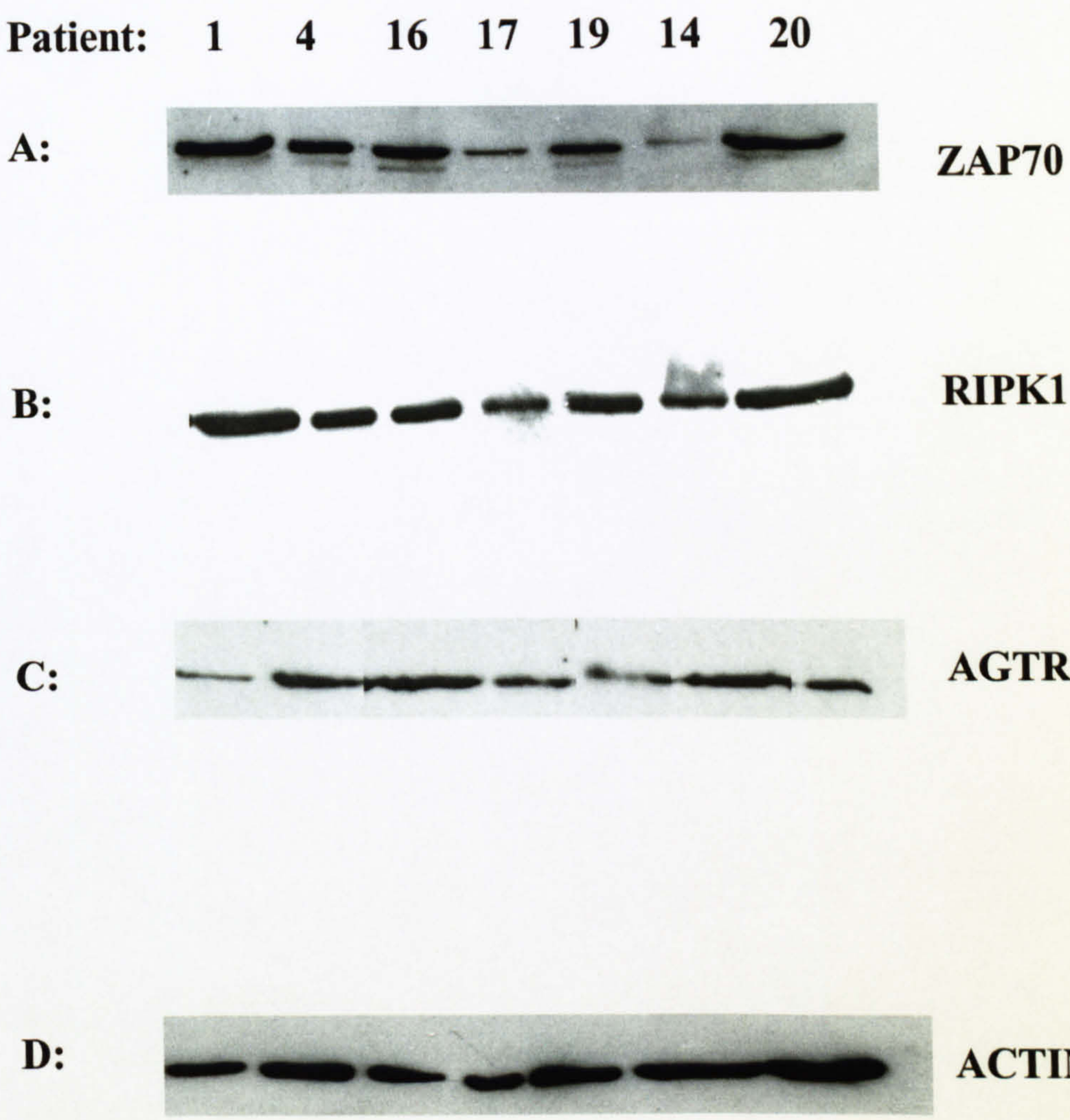


Figure 6.4 Comparison of RIPK1 and AGTR1 protein levels with ZAP70. Cell lysates were made from B-CLL cells (patients 1, 4, 16, 17, 19, 14 and 20) and separated by SDS PAGE. The gels were then immunoblotted and probed with specific antibodies to ZAP70 (A), RIPK1 (B), AGTR1 (C) and actin (D). RIPK1 protein levels correlated with ZAP70 levels but AGTR1 did not. Each Western was performed once.

rearrangement is initiated, by first joining D_H (diversity) to J_H (joining) genes, then V_H (variable) genes to DJ_H genes and IGLJ3 is a joining segment. This leads to synthesis of a heavy chain which associates with a surrogate light chain to form the preB cell receptor (preBCR) (Hardy and Hayakawa, 2001; Rolink et al., 2001). IGLL1 encodes a surrogate light chain for the preBCR. Correct assembly of the preBCR and signalling from the receptor allow B cell development to proceed to the next stage, the preB cell and induces proliferation of the cells. In addition heavy chain recombination is turned off so that only one heavy chain is synthesised. Surrogate light chain expression is also turned off and light chain rearrangement up-regulated leading to production of IgM, the B cell receptor. If IGLL1 is still expressed in ZAP70+ patients this suggests that they may still have preBCR formed. It has been reported that ZAP70 is expressed in B cells throughout their development, and has also been shown to have role in signalling from the preBCR (Schweighoffer et al., 2003).

INHBA (inhibin beta A or ßA) is a subunit for molecules of the activin family. ßA can form a homodimer designated activin A, or a heterodimer with another activin family member ßB to form activin AB. It can also associate with inhibinα to form inhibin, which antagonizes the actions of activins. In order for a cell to produce the inhibin heterodimer without concurrent activin production, the α subunit must be produced in excess of the ß subunit (Shav-Tal and Zipori, 2002). Activin A has a wide range of activities, including a role in the regulation of haematopoiesis. It is an erythroid differentiation factor and is expressed in the bone marrow microenvironment, although not usually in B cells (Yu et al., 1987; Shiozaki et al., 1992; Murata et al., 1988). There is evidence that activin A may play a role in bone marrow B lymphopoiesis. It is known that B lymphopoiesis requires interactions with the bone marrow stroma (Grawunder et al., 1993; Veiby et al., 1997) and it has been proposed that activin A may play an inhibitory role in B lymphopoiesis. Suppression of activin A action in long term bone marrow lymphopoietic cultures resulted in the early onset of B lymphopoiesis (Shoham and Zipori, 1998). Moreover *in vivo*, a specific shut off of activin A was observed in spleen and bone marrow of mice following the induction of B-cell polyclonal activation (Shoham et al., 2003). Therefore, expression of activin A in cells of ZAP70+ B-CLL patients could block B cell development.

MTR (5-methyltetrahydrofolate-homocysteine methlytransferase), or methionine synthase, catalyses endogenous methionine synthesis by re-methylation of homocysteine, in the presence of 5-methyltetrahydrofolate as a methyl donor and colbalamin (vitamin B12) as a cofactor (Banerjee and Matthews, 1990). MTR expression can be regulated by methionine, colbalamin and homocysteine. Regulation is important for the level of methionine synthesis. Methionine is the precursor of S-adenosyl-methionine, the methyl donor for methylation of nucleotides, proteins and lipids. Aberrant DNA methylation can play a significant role in tumorigenesis (Jones and Baylin, 2002; Ehrlich, 2002). This has been observed in B-CLL (Issa et al., 1997) and was found to correlate with I_gV_H mutational status (Lyko et al., 2004). High levels of DNA methylation were observed in B-CLL patients with unmutated I_gV_H genes and therefore might correlate with increased polysome association of MTR. Regulation of MTR expression by colbalamin has been shown to occur at the translation level. It increases translation of MTR by binding to colbalamin response elements in the 5'UTR of MTR mRNA (Gulati et al., 1999; Oltean and Banerjee, 2003).

6.4 Comparison of ZAP70 status of the B-CLL patients with the I_gV_H gene mutational status

The I_gV_H gene mutational status for the B-CLL patients (where available) was subsequently obtained (provided by Prof. Martin Dyer) and compared to the ZAP70 status (Table 6.1). The ZAP70 status correlated with the immunoglobulin mutational status for the majority of patients, with just two exceptions. Patient 11 had mutated I_gV_H genes, as was predicted from the microarray data in Figure 6.3. However, patient 9 was also observed to have mutated I_gV_H genes. Not only has ZAP70 been identified as a prognostic marker for B-CLL, but there is now evidence that suggests it may have a functional role. B-CLL cells vary in their capacity to respond to IgM ligation and this was shown to correlate with I_gV_H mutational status (Chen et al., 2002; Lanham et al., 2003). This increased signalling has been shown to be due to higher ZAP70 protein levels, which has been observed to directly enhance BCR receptor signalling (Chen et al., 2002, 2004). In fact, it was observed that BCR signalling capacity correlated with ZAP70 levels rather than with I_gV_H gene mutation status (Chen et al., 2004), which suggests that this could be a more reliable prognostic marker for disease progression. Therefore, the genes identified may show a correlation specifically with ZAP70 status rather than I_gV_H mutational status, and patient 9 may have a

gene expression profile that correlates with its ZAP70 status rather than its mutational status.

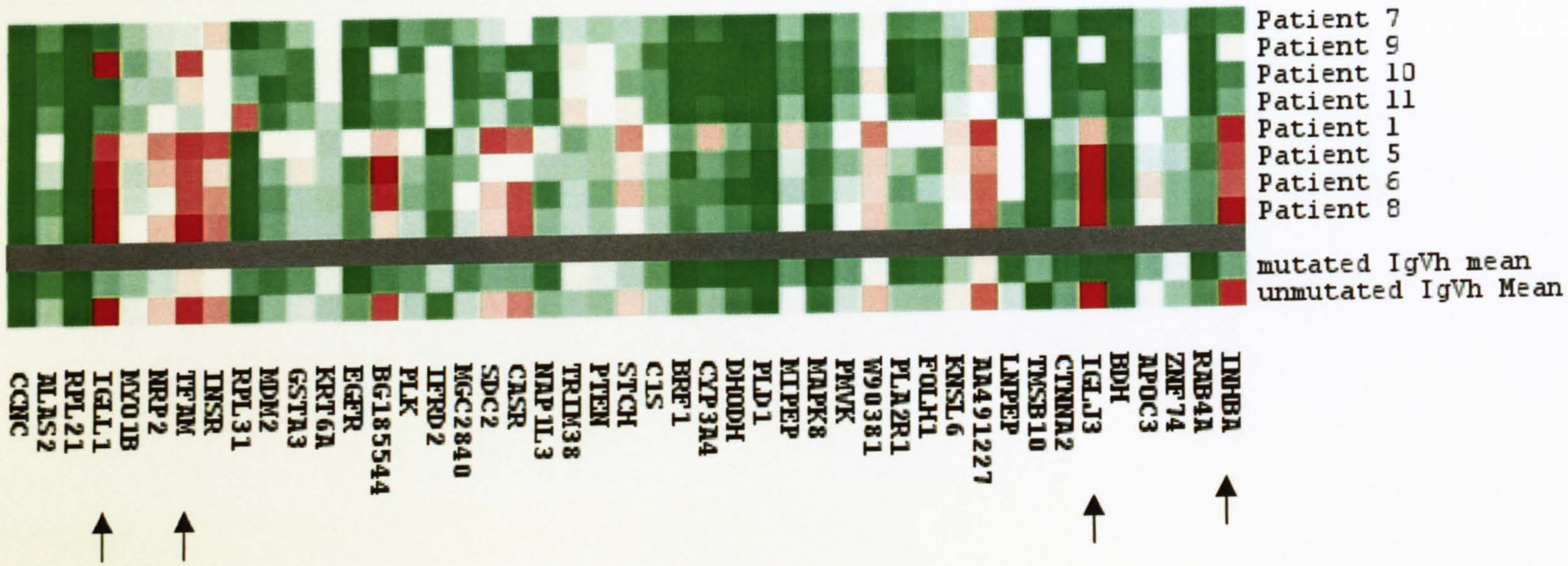
Patient	Zap70 status	VH homology (%)
1	Positive	99
2	Positive	Unknown
3	Unknown	Unknown
4	Positive	Unknown
5	Positive	100
6	Positive	99
7	Negative	96
8	Positive	99
9	Positive	97
10	Negative	96
11	Positive	96

Table 6.1 Zap70 status and VH mutation status for patients used in the microarray analysis.

6.5 Microarray analysis of translation comparing patients depending upon their I_gV_{II} gene mutational status

The patients were grouped based on I_gV_H gene mutation status and microarray data compared, to identify differences in gene expression at the level of translation between the two subtypes. 124 genes were identified with differential polysome association; 45 genes had higher polysome association in patients with mutated I_gV_{II} genes (Figure 6.5 and Appendix 10) and 74 higher in patients with unmutated I_gV_H genes (Figure 6.6 and Appendix 11). The overall number of significantly different genes was lower than the number produced when comparing patients with different ZAP70 status. This was probably due to a more even number of patients in each subgroup (four in each), which may have

Figure 6.5 Genes with higher polysome association in B-CLL patients with mutated I_gV_H . A colour scale has been used to represent the ratio of subpolysome to polysome message for each B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated. Patients 7, 9, 10 and 11 had mutated I_gV_H genes and patients 1, 5, 6 and 8 unmutated. Those genes which are of particular interest are highlighted by arrows.


Patient 7
Patient 9
Patient 10
Patient 11
Patient 1
Patient 5
Patient 6
Patient 8
mutated IgVh mean
unmutated IgVh Mean
CCNC
ALAS2
RPL21
IGLL1
MYO1B
NRP2
TFRM
INSR
RPL31
MDM2
GSTA3
KRT6A
EGFR
BG185544
PLK
IFRD2
MGC2840
SDC2
CASR
NAP1L3
TRIM38
PTEN
STCH
C1S
BRF1
CYP3A4
DHODH
PLD1
MIPEP
MAPK8
PMVK
W90381
PLA2R1
FOLH1
KNSL6
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LNPEP
TMSB10
CTNNA2
IGLJ3
BDH
APOC3
ZNF74
RAB4A
INHBA

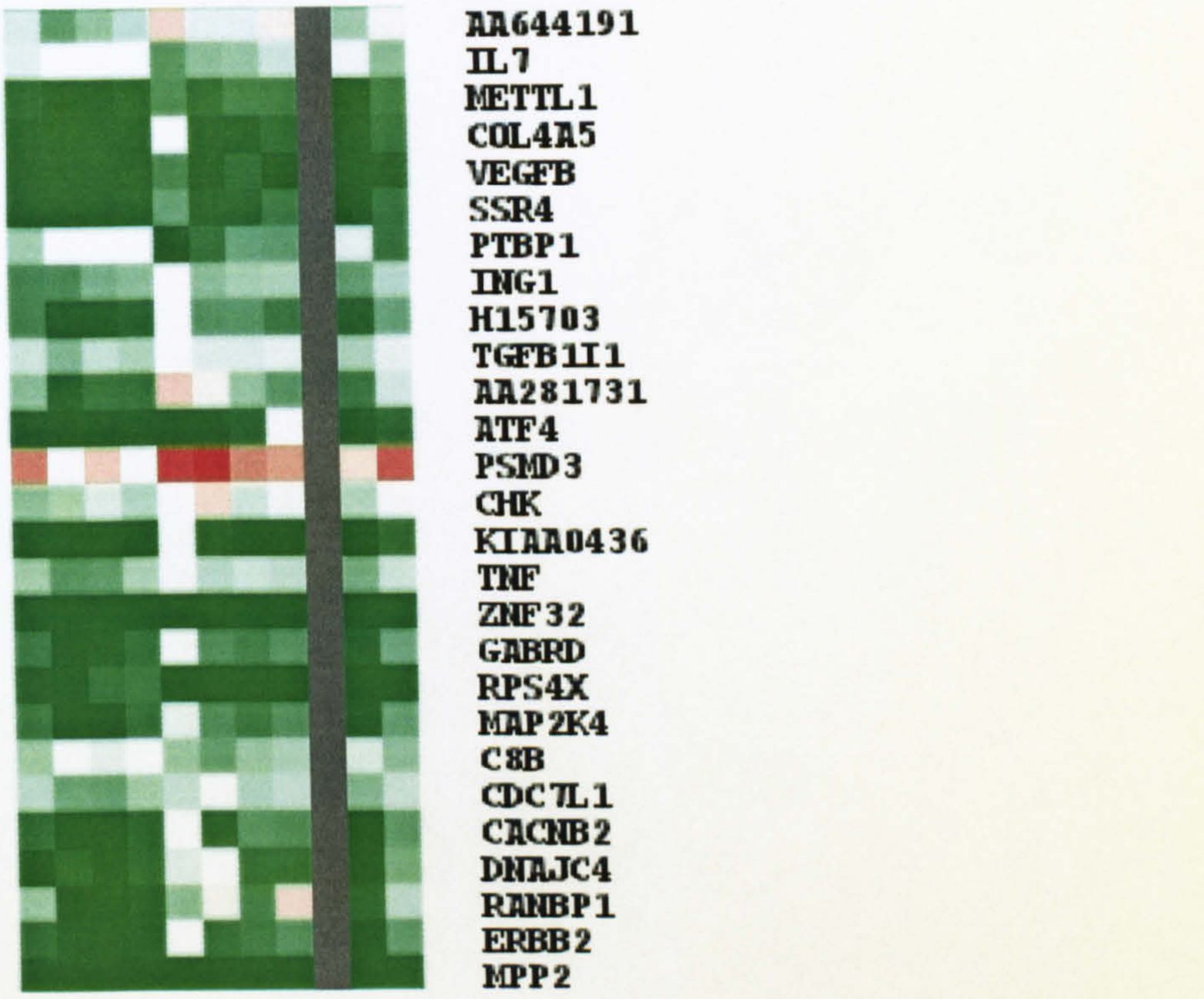


Figure 6.6 Genes with higher polysome association in B-CLL patients with unmutated I_gV_H genes. A colour scale has been used to represent the ratio of subpolysome to polysome message for each B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated. Patients 7, 9, 10 and 11 had mutated I_gV_H genes and patients 1, 5, 6 and 8 unmutated. Those genes which are of particular interest are highlighted by arrows.

reduced the number of differences identified due to individual patient variation. Inspection of the data reveals that the group of genes highlighted above, with very distinct differences in polysome association dependent of ZAP70 status discussed above (Figure 6.3), were all found to be significantly different depending on I_gV_H gene mutational status, with the exception of MTR and caspase 1 (Figure 6.6).

Interestingly, Syk was observed to have higher polysome association in patients with mutated I_gV_H genes (Figure 6.5). Syk is a protein tyrosine kinase involved in BCR signalling, as previously explained, and phosphorylates a number of downstream targets in the signalling pathway (Takata et al., 1994; Ishiai et al., 1999; Kabak et al., 2002). This leads to a variety of responses, including calcium mobilisation. Syk protein levels have previously been observed to be normal in most cases of B-CLL (Semichon et al., 1997; Chen et al., 2002) however they were found to be variable in another report (Lankester et al., 1995). The increased ZAP70 levels in patients with unmutated I_gV_H genes has been shown to increase BCR signalling as described above, and would therefore compensate for lower levels of Syk. Syk also has an important role in signalling from the preBCR. However, as mentioned above, in the absence of syk, development in mice was only partially blocked (Turner et al., 1995; Cheng et al., 1995) and ZAP70 was shown to be able to signal through the preBCR instead (Schweighoffer et al., 2003). A recent study has observed that syk deficient mice were unable to undergo light chain rearrangement (Meade et al., 2004), which could explain the higher polysome association of IGLL1 that was observed for these patients.

AMD1 (S-adenosylmethionine decarboxylase 1) was also observed to have higher polysome association in patients with mutated I_gV_H genes (Figure 6.5). It is a key enzyme involved in polyamine biosynthesis. Polyamines are required for cell growth and differentiation and have been implicated in a number of cellular processes including DNA replication, transcription and translation (Marton and Morris, 1991; Morris, 1991). Optimal levels are required for growth and differentiation therefore there are numerous regulatory mechanisms exerted. Polyamines and their biosynthetic machinery are generally observed to be up-regulated in rapidly growing cells and cancer cells, compared to quiescent cells (Scalabrino and Ferioli, 1981; Seiler et al 1990). In contrast, depletion of polyamines has been observed to cause cell cycle arrest and apoptosis (Schipper et al, 2000; Nitta et al,

2002). The biosynthetic pathway consists of two decarboxylases, which are highly regulated, AMD1 and ornithine decarboxylase (ODC), plus spermidine synthase and spermine synthase, which are constitutively expressed. AMD1 catalyses the decarboxylation of *S*-adenosylmethionine, generating the *N*-propylamine donor required for the conversion of putrescine to spermidine, catalysed by spermidine synthase. Putrescine is produced by the decarboxylation of ornithine, catalysed by ODC. AMD1 and ODC are both subject to regulation at multiple levels of gene expression, including translation. Spermidine and spermine have been observed to repress translation of AMD1 by causing ribosomes to stall at the termination codon of a uORF in the 5'UTR of AMD1 mRNA, thus blocking the scanning of additional ribosomes and therefore translation of the coding ORF (Ruan et al, 1996; Mize et al, 1998; Law et al, 2001; Raney et al, 2002). ODC mRNA has been observed to contain an IRES element in its 5'UTR (Pyronnet et al., 2000). ODC and spermidine synthase (SRM) were both observed to have decreased polysome association in B-CLL patients compared to CD19+ B cells (Appendix 4), suggesting that polyamine synthesis is reduced in all B-CLL patients irrespective of subtype and AMD1 levels, and correlates with the quiescent phenotype of B-CLL cells. Moreover, *S*-adenosylmethionine, the substrate for AMD1, is also the substrate for methylation reactions, including DNA methylation. Therefore, reduced levels of AMD1 could lead to an increase in *S*-adenosylmethionine and potentially an increase in DNA methylation. DNA methylation has been observed to be elevated in B-CLL patients with unmutated I_gV_H genes (Lyko et al., 2004), and therefore correlates with reduced AMD1 polysome association.

6.6 Discussion

The data produced by the cDNA microarray analysis of translation in B-CLL cells was analysed to determine if there were any differences due to the I_gV_H gene mutation status. ZAP70 protein levels have been previously observed to correlate with I_gV_H gene mutation status in B-CLL (Crespo et al., 2003) therefore western analysis was performed to determine ZAP70 expression in the B-CLL patients used for the microarray analysis presented in chapter 4. This identified patients 7 and 10 as having low ZAP70 protein levels and therefore assumed to have mutated I_gV_H genes, and the rest high ZAP70 levels with unmutated I_gV_H genes. Thus polysome association for the two groups was compared and identified 239 genes with differential polysome association. 125 genes had higher polysome association in patients with low ZAP70 levels, and 114 higher in patients with

high ZAP70 levels. However, many of the differences in polysome association observed may have been a result of individual patient variation due to the low number of patients used in the analysis. Western analysis was performed to determine if two genes identified in the microarray analysis correlated with ZAP70 protein levels, using a separate set of patients not used in the microarray analysis (patients 14, 16, 17 and 20) plus two that were (patients 1 and 4). RIPK1 was observed to have lower polysome association in B-CLL patients with low ZAP70 levels. Western analysis revealed a correlation with ZAP70 protein levels, suggesting that the results observed in the microarray analysis were correct for this gene. However AGTR1, which was observed to have higher polysome association in patients with low ZAP70, did not show any correlation when Western analysis was performed with the second set of patients, suggesting that the variation observed may be individual or may correlate with another prognostic factor.

There were a number of genes observed to have a very distinct difference in polysome association between the two subtypes, all having lower polysome association in B-CLL patients with low ZAP70 levels. This suggested that these differences may be accurate and may have a significant effect on the biology of the disease. Differential expression of MTR may affect the methylation levels in B-CLL cells, which have been observed to be higher in patients with unmutated I_gV_H genes (Lyko et al., 2004). MTR catalyses endogenous biosynthesis of methionine, the precursor of *S*-adenosylmethionine which is the methyl donor for methyation of DNA. In addition, two of the genes suggest a link with B cell development. IGLL1 is a surrogate light chain for the preBCR, whose signalling is required in B cell development. Interestingly evidence has suggested that ZAP70 may function in preB cell signalling (Schweighoffer et al., 2003). Higher expression in B-CLL ZAP70 positive B cells of INHBA could result in increased levels of a homodimer of this protein, activin A. There is evidence that activin A has a negative role in B cell development, as suppression of this protein has been shown to result in early onset of B lymphopoiesis (Shoham, 1998). Therefore expression of activin A could be hypothesized to block B cell development in B-CLL cells.

The polysome association observed for this group of genes for patient 11 correlated with the ZAP70 low patients, despite a high level of ZAP70 being observed. ZAP70 protein levels do not correlate 100% with the I_gV_H gene mutation status, therefore this patient may

have been placed in the wrong group. However, there is evidence that ZAP70 may be a prognostic factor itself, as it has been observed that ZAP70 can enhance BCR signalling (Chen et al., 2002; Chen et al., 2004). Subsequently, the I_gV_H gene mutation status for the B-CLL patients used in the microarray study was obtained. This revealed that patient 11 had been placed in the wrong group and did have mutated I_gV_H genes. However, this was also the case for patient 9. The microarray data was re-analysed, grouping the patients using I_gV_H gene mutation status. 124 genes were identified with differential polysome association; 45 genes had higher polysome association in patients with mutated I_gV_H genes and 79 higher in patients with unmutated I_gV_H genes. Of those genes observed to have a very distinct difference in polysome association depending on ZAP70 protein levels, all but MTR and caspase 1 were also found to have the same difference when I_gV_H gene mutation status was used. Syk was observed to have higher polysome association in patients with mutated I_gV_H genes, which may be of significance if correct, due to its important role in BCR signalling. Another gene of particular interest with differential polysome association was AMD1, which had lower polysome association in patients with mutated I_gV_H genes. Like MTR, differential expression of this gene may have an effect on DNA methylation levels as a consequence of its role in the metabolism of *S*-adenosylmethionine.

Chapter 7
Discussion

7.1 Translation regulation in B-CLL

Translation regulation has become recognised as an important process for the control of gene expression, and de-regulation in cancer has been observed to be significant in numerous examples. Translation regulation has not previously been studied for chronic lymphocytic leukaemia however experiments performed in this study indicate that it may be important. The results obtained suggest that a reduced rate of protein synthesis was occurring in B-CLL cells, consistent with the quiescent phenotype that is observed (Andreeff et al., 1980; Caligaris-Cappio and Hamblin, 1999). Differential expression of a subset of messages was subsequently revealed by the translation profile produced using cDNA microarray analysis, which also had good correlation with the biology of the disease.

7.2 Translation status of B-CLL cells

Experiments were performed to determine the translational status of B-CLL cells and identify any global translation defects. Results revealed that a reduced level of protein synthesis was occurring in B-CLL cells. A decrease in polysome associated messages was observed for B-CLL cells compared to GM1953 and tonsil CD19+ B cells, which could be due to reduced levels of eIF4E as observed by Western analysis. B-CLL cells have previously been found to be quiescent (Andreef et al., 1980; Caligaris-Cappio and Hamblin, 1999) and therefore would not be expected to have high translation rates. This was further reflected by the presence of 5'TOP messages in the subpolysome fractions for B-CLL cells, a group of genes translationally up-regulated in response to growth signals. No defects were identified therefore the reduced level of translation suggested by the data may be a consequence of the quiescent state of B-CLL cells rather than a cause. Variation in eEF2 and 4EBPI levels were observed for a subset of patients whose RNA was found to be degraded when polysome analysis was performed. This was subsequently revealed to occur during preparation of samples and was probably due to the presence of a highly active RNase. The variation in translation factor levels may correlate with findings of a previous study, which defined two subgroups of B-CLL by expression of translation and ribosome associated genes (Dürig et al., 2003). Further analysis would be required to

determine if this was the case and the RNase activity would need to be overcome for further analysis of translation in this subset.

cDNA microarray analysis was performed to produce a translation profile for B-CLL cells, using the proportion of polysome associated message as a measure of the level of translation for each gene. Primary CD19+ B cells, isolated from tonsil samples from healthy volunteers were used as a control. This cell type is not perfect for use as control as it is not the cell of origin for B-CLL, so differences in gene expression identified may reflect this fact rather than being significant to disease biology. Those genes identified however, in general show good correlation with the biology of the disease, suggesting that the results obtained may be significant. A number of genes that could be associated with the anti-apoptotic phenotype were observed to have differential polysome association in B-CLL cells, such as Bcl2, UCP2 and PDCD4. There were also genes that could be involved in survival signalling including CXCR4, STAT1 and various interleukins. The quiescent status of B-CLL cells could be influenced by levels of D type cyclins, or the increased polysome association of BTG1 and WEE1. The changes in translation identified may be suggested to only reflect changes observed at the level of transcription. However, a comparison with microarray analysis of transcription in B-CLL revealed only a small fraction of genes to show significant up or down-regulation of expression for both processes. Taken together the results obtained suggest translation regulation is important for the biology of B-CLL. Moreover, it highlights the importance of analysing gene expression at the level of translation in general and suggests that production of translation profiles, using microarray analysis, could be useful for the study of other types of cancer.

Translational profiling for B-CLL cells was also used to analyse differences between the two subtypes of B-CLL, defined by I_gV_H gene mutation status. This was performed using the microarray data for B-CLL patients already obtained, comparing polysome association of mRNAs for patients from each subtype. The analysis was performed twice, using ZAP70 as well as I_gV_H gene mutation status to define the subtypes. ZAP70 does not correlate 100% with I_gV_H gene mutation as is the case for this set of patients. The lists of genes produced, using each marker, were similar although some differences were observed. However, as only a small patient sample for this kind of analysis was available, data obtained may only reflect differences due to individual patient variation rather than a consequence of I_gV_H gene mutation status. ZAP70 has recently been suggested to correlate

with BCR signalling capacity in B-CLL (Chen et al., 2004), therefore differences in gene expression identified using this disease marker could be of biological significance. A number of genes were observed to have a very distinct difference in polysome association, suggesting they could potentially be significant. The function of some of the genes could be hypothesised to have a role in the differences between the two subtypes that are observed. INHBA, in its homodimer form activin A, has a role in B cell development, which could affect differences between the two subtypes. IGLL1 and Syk may affect the differential BCR signalling observed between the subtypes (Chen et al., 2002; Lanham et al., 2003). Whilst, differential expression of MTR and AMD1 may affect the level of DNA methylation, previously observed to be different between the two subtypes (Lyko et al., 2004). Further analysis, with a larger number of patients, is required to determine differences between the two subtypes accurately. However, the initial results obtained provide evidence that again translation regulation may be important.

7.3 Mechanism of translation regulation

There are a variety of mechanisms utilised for translation regulation, as discussed in section 1.4, which could be used by those messages identified to have differential translation in B-CLL cells. Experiments performed provide evidence that internal ribosome entry could be a mechanism of regulation used. Accumulating evidence has suggested that the use of IRES mediated translation has an important role in the regulation of expression of certain genes. These are often genes which encode proteins involved in the control of cell growth and apoptosis, processes often deregulated in tumorigenesis. IRES activity was observed for three mRNAs found to have increased polysome association by the microarray analysis, BTG1, UCP2 and CSNK2B and this activity was maintained under conditions of serum starvation. As serum starvation causes growth arrest, the conditions created may be similar to that in B-CLL cells thereby suggesting IRES mediated translation might be used. More evidence is required to determine whether IRES mediated translation is used by these three mRNAs in B-CLL cells. A number of control experiments must be performed initially, to confirm that IRES mediated translation was responsible for the data obtained, as there are alternative explanations such as splicing or cryptic promoters that could also account for the results. More mRNAs identified by the microarray analysis should be tested for the presence of IRES elements to determine if this may be a common mechanism of regulation used. Plus, IRES activity could be analysed in B cells under normal and growth arrest

conditions, and perhaps in a B-CLL mouse model to provide stronger evidence for use of internal ribosome entry in B-CLL cells.

Other, mechanisms of translation regulation could also be used in B-CLL. MicroRNAs have recently emerged as important regulators of translation and could have a role in B-CLL. Two microRNAs have been found in the 13q14 chromosome region, which is often deleted in B-CLL (Calin et al., 2002). MiRNAs repress translation of target mRNAs therefore deletion may relieve repression of genes significant to the biology of the disease. In a recent study, a profile of miRNA expression for B-CLL cells was produced (Calin et al., 2004). It was observed that a number of miRNAs were differentially expressed in B-CLL cells, and between B-CLL subtypes so may affect gene expression. The mechanism of translation repression used by miRNAs has not been deduced, although they might act at the elongation or termination stage of translation as it was observed that repression of *lin-14* translation by the miRNA *lin-4* did not alter the association of the message with polysomes (Olsen and Ambros, 1999). If this is the case miRNA regulation of translation would not be identified by the microarray analysis performed in this study. However, it is not known if this is the actual mechanism of regulation, or if it is widely used.

7.4 Functional significance of translation regulation in B-CLL

If the changes in translation identified are reflected at the protein level they might have an effect on B-CLL cell biology and therefore may represent new targets for treatment. As B-CLL cells are resistant to apoptosis *in vivo* but spontaneously apoptose *in vitro* (Collins et al., 1989; MacFarlane et al., 2002), they are thought to require survival signals from their microenvironment for survival. Up-regulation of a receptor for such a survival signal may therefore increase signalling. Data obtained provides evidence that the receptor AGTR1 may function in B-CLL cell survival *in* vivo. AGTR1 was observed to have increased polysome association in the microarray analysis, plus an increase in protein levels in B-CLL cells. The ligand for AGTR1, angiotensin II was observed to reduce spontaneous apoptosis when freshly isolated B-CLL cells were cultured *in vitro.* This effect was not observed for tonsil CD19+ B cells, suggesting that it was specific for B-CLL cells. The reduction in apoptosis was quite small however, although consistently observed. It was hypothesised that angiotensin II might be found in the serum used in the growth medium and thus saturate AGTR1 receptors reducing the effect observed. Further analysis would be required to determine if the effect was significant. However, if found

to be real, AGTR1 could become a new target for treatment. Drugs have previously been developed that specifically target the AGTR1 receptor, for use in treating hypertension, therefore are already available if the effect was proven to be significant.

Other genes identified in the microarray analysis might also have an important effect on the biology of the disease, as suggested by their normal functions, and analysis might provide useful new information about B-CLL biology. Moreover, the extensive number of new targets identified again highlights the potential importance of translation regulation in B-CLL as well as other types of cancer.

Appendix 1

1.1 Table of genes with significantly increased polysome association in B-CLL cells when compared to GM1953 cells.

Cluster Number	Gene Name	Gene Description
Hs.91065	RBBP6	retinoblastoma binding protein 6
Hs.180877	H3F3B	H3 histone, family 3B (H3.3B)
Hs.155485	HIP2	huntingtin interacting protein 2
Hs.171626	SKP1A	S-phase kinase-associated protein 1A (p19A)
Hs.10758	NDUFS4	NADH dehydrogenase (ubiquinone) Fe-S protein 4, 18kDa (NADH-coenzyme Q reductase)
Hs.159161	ARHGDIA	Rho GDP dissociation inhibitor (GDI) alpha
Hs.77054	BTG1	B-cell translocation gene 1, anti-proliferative
Hs.9964	MRPS12	mitochondrial ribosomal protein S12
Hs.80395	MAL	mal, T-cell differentiation protein
Hs.355779	AA630507	Homo sapiens clone TCCCTA00211 mRNA sequence
Hs.77558	HMGN3	high mobility group nucleosomal binding domain 3
Hs.81424	UBL1	ubiquitin-like 1 (sentrin)
Hs.180139	SMT3H2	SMT3 suppressor of mif two 3 homolog 2 (yeast)
Hs.76362	GTF2A2	general transcription factor IIA, 2, 12kDa
Hs.191887	SEC61B	protein translocation complex beta
Hs.82425	ARPC5	actin related protein 2/3 complex, subunit 5, 16kDa
Hs.315054	MGC15875	hypothetical protein MGC15875
Hs.315463	IL24	interleukin 24
Hs.184488	FLOT2	flotillin 2
Hs.89472	AGTR1	angiotensin II receptor, type 1
Hs.889	CLC	Charot-Leyden crystal protein
Hs.279574	GRIM19	cell death-regulatory protein GRIM19
Hs.256697	HINT1	histidine triad nucleotide binding protein 1
Hs.192861	SPIB	Spi-B transcription factor (Spi-1/PU.1 related)
Hs.85119	SMT3H1	SMT3 suppressor of mif two 3 homolog 1 (yeast)
Hs.380080	FKBP1A	FK506 binding protein 1A, 12kDa
Hs.374993	AA076063	Homo sapiens, clone IMAGE:4296901, mRNA, mRNA sequence
Hs.77252	FHIT	fragile histidine triad gene
Hs.293750	ARPC3	actin related protein 2/3 complex, subunit 3, 21kDa
Hs.356785	AA193254	ESTs, Highly similar to IF4E_HUMAN Eukaryotic translation initiation factor 4E (eIF-4E) (eIF4E) (mRNA cap-binding protein) (eIF-4F 25 kDa subunit) [H.sapiens]
Hs.348412	TCF7L2	transcription factor 7-like 2 (T-cell specific, HMG-box)
Hs.77496	SNRPG	small nuclear ribonucleoprotein polypeptide G
Hs.1087	NEK4	NIMA (never in mitosis gene a)-related kinase 4
Hs.75528	HUMAUANTIG	nucleolar GTPase
Hs.519	WWOX	WW domain containing oxidoreductase
Hs.406384	CBX3	chromobox homolog 3 (HP1 gamma homolog, Drosophila)
Hs.58685	CD5	CD5 antigen (p56-62)
Hs.75104	RNPS1	RNA binding protein S1, serine-rich domain
Hs.168913	STK24	serine/threonine kinase 24 (STE20 homolog, yeast)
Hs.155103	EIF1AY	eukaryotic translation initiation factor 1A, Y chromosome
Hs.119222	ST13	suppression of tumorigenicity 13 (colon carcinoma) (Hsp70 interacting protein)
Hs.34074	DPP6	dipeptidylpeptidase VI
Hs.236361	RNPC1	RNA-binding region (RNP1, RRM) containing 1

Hs.103042	MAP1B	microtubule-associated protein 1B
Hs.170414	PACE4	paired basic amino acid cleaving system 4
Hs.349650	RAF1	v-raf-1 murine leukemia viral oncogene homolog 1
Hs.75789	NDRG1	N-myc downstream regulated gene 1
Hs.180714	COX6A1	cytochrome c oxidase subunit VIa polypeptide 1
Hs.412292	R39428	ESTs, Moderately similar to protein tyrosine phosphatase, receptor type, G; protein tyrosine phosphatase, receptor type, gamma polypeptide [Homo sapiens] [H.sapiens]
Hs.181243	ATF4	activating transcription factor 4 (tax-responsive enhancer element B67)
Hs.10882	HBP1	HMG-box containing protein 1
Hs.74002	NCOA1	nuclear receptor coactivator 1
Hs.105465	SNRPF	small nuclear ribonucleoprotein polypeptide F
Hs.183583	SERPINB1	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1
Hs.336628	RPL36AL	ribosomal protein L36a-like
Hs.349506	PC4	activated RNA polymerase II transcription cofactor 4
Hs.391800	AKR1C-pseudo	pseudo-chlordecone reductase
Hs.381152	GRB2	growth factor receptor-bound protein 2
Hs.88646	DNASE1L3	deoxyribonuclease I-like 3
Hs.45002	RAC3	ras-related C3 botulinum toxin substrate 3 (rho family, small GTP binding protein Rac3)
Hs.12109	CIAO1	WD40 protein Ciao1
Hs.78589	SERPINI1	serine (or cysteine) proteinase inhibitor, clade I (neuroserpin), member 1
Hs.433562	MYL4	myosin, light polypeptide 4, alkali; atrial, embryonic
Hs.268571	APOC1	apolipoprotein C-I
Hs.865	RAP1A	RAP1A, member of RAS oncogene family
Hs.323502	NXF1	nuclear RNA export factor 1
Hs.10082	KCNN4	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4
Hs.7645	FGB	fibrinogen, B beta polypeptide
Hs.334841	SELENBP1	selenium binding protein 1
Hs.109918	ARHH	ras homolog gene family, member H
Hs.74597	STIM1	stromal interaction molecule 1
Hs.406269	STMN1	stathmin 1/oncoprotein 18
Hs.194382	ATM	ataxia telangiectasia mutated (includes complementation groups A, C and D)
Hs.300697	IGHG3	immunoglobulin heavy constant gamma 3 (G3m marker)
Hs.83393	CST6	cystatin E/M
Hs.82422	CAPG	capping protein (actin filament), gelsolin-like
Hs.174031	COX6B	cytochrome c oxidase subunit VIb
Hs.155418	GS3955	GS3955 protein
Hs.44313	REL	v-rel reticuloendotheliosis viral oncogene homolog (avian)
Hs.115907	DGKD	diacylglycerol kinase, delta 130kDa
Hs.429443	N53664	ESTs, Highly similar to C5HU complement C5 precursor - human [H.sapiens]
Hs.372864	N62562	Similar to uterine protein [Homo sapiens], mRNA sequence
Hs.2706	GPX4	glutathione peroxidase 4 (phospholipid hydroperoxidase)
Hs.75410	HSPA5	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)
Hs.431520	AA777406	ESTs, Moderately similar to roundabout 1, isoform a; axon guidance receptor [Homo sapiens] [H.sapiens]
Hs.113882	GABRD	gamma-aminobutyric acid (GABA) A receptor, delta
Hs.423605	N94385	EST, Weakly similar to COMP_HUMAN Cartilage oligomeric matrix protein precursor (COMP) [H.sapiens]
Hs.75648	PLCG2	phospholipase C, gamma 2 (phosphatidylinositol-specific)
Hs.350899	AA102454	Homo sapiens cDNA FLJ30484 fis, clone BRAWH2000071, mRNA

		sequence
Hs.90708	GZMA	granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)
Hs.367740	HSPC022	HSPC022 protein
Hs.79769	PCDH1	protocadherin 1 (cadherin-like 1)
Hs.75576	PLG	plasminogen
Hs.184276	SLC9A3R1	solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulatory factor 1
Hs.82568	CYP27A1	cytochrome P450, subfamily XXVIIA (steroid 27-hydroxylase, cerebrotendinous xanthomatosis), polypeptide 1
Hs.91747	PFN2	profilin 2
Hs.248	MAP3K8	mitogen-activated protein kinase kinase kinase 8
Hs.109918	ARHH	ras homolog gene family, member H
Hs.101370	Z43075	Homo sapiens full length insert cDNA clone ZC64D04, mRNA sequence
Hs.272493	CCL15	chemokine (C-C motif) ligand 15
Hs.74368	CKAP4	cytoskeleton-associated protein 4
Hs.155396	NFE2L2	nuclear factor (erythroid-derived 2)-like 2
Hs.347508	BG185544	Homo sapiens cDNA: FLJ23482 fis, clone KAIA03142, mRNA sequence
Hs.366	PTS	6-pyruvoyltetrahydropterin synthase
Hs.47626	RIT2	Ras-like without CAAX 2
Hs.211600	TNFAIP3	tumor necrosis factor, alpha-induced protein 3
Hs.168669	OGDH	oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)
Hs.80315	SH3GL3	SH3-domain GRB2-like 3
Hs.104555	NPFF	neuropeptide FF-amide peptide precursor
Hs.24976	ART3	ADP-ribosyltransferase 3
Hs.79706	PLEC1	plectin 1, intermediate filament binding protein 500kDa
Hs.181304	13CDNA73	hypothetical protein CG003
Hs.20716	TIMM17A	translocase of inner mitochondrial membrane 17 homolog A (yeast)
Hs.287797	ITGB1	integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
Hs.12045	C2F	C2f protein
Hs.78281	RGS12	regulator of G-protein signalling 12
Hs.272003	HBZ	hemoglobin, zeta
Hs.30888	COX7A2L	cytochrome c oxidase subunit VIIa polypeptide 2 like
Hs.240013	COMT	catechol-O-methyltransferase
Hs.130829	HSU53209	transformer-2 alpha (htra-2 alpha)
Hs.383241	N39380	ESTs, Moderately similar to deleted in lymphocytic leukemia, 2; leukemia associated gene 2 [Homo sapiens] [H.sapiens]
Hs.82527	SIAT8A	sialyltransferase 8A (alpha-N-acetylneuraminate: alpha-2,8-sialytransferase, GD3 synthase)
Hs.74573	PLD3	likely ortholog of mouse phospholipase D3
Hs.32916	NACA	nascent-polypeptide-associated complex alpha polypeptide
Hs.81008	FLNB	filamin B, beta (actin binding protein 278)
Hs.75207	GLO1	glyoxalase I
Hs.278857	HNRPH2	heterogeneous nuclear ribonucleoprotein H2 (H')
Hs.85092	TRIP11	thyroid hormone receptor interactor 11
Hs.75770	RB1	retinoblastoma 1 (including osteosarcoma)
Hs.8136	DPP7	dipeptidylpeptidase 7
Hs.1244	CD9	CD9 antigen (p24)
Hs.76751	SDS	serine dehydratase
Hs.169266	NPY1R	neuropeptide Y receptor Y1
Hs.82401	CD69	CD69 antigen (p60, early T-cell activation antigen)
Hs.78225	ANXA1	annexin A1
Hs.433402	CFDP1	craniofacial development protein 1
Hs.75428	SOD1	superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))

Hs.77385	MYL6	myosin, light polypeptide 6, alkali, smooth muscle and non-muscle
Hs.432567	AA858296	ESTs, Highly similar to NPT4_HUMAN Sodium-dependent phosphate transport protein 4 (Sodium/phosphate cotransporter 4) (Na(+)/PI cotransporter 4) [H.sapiens]
Hs.172195	MGAT2	mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase
Hs.433543	N49703	ESTs, Moderately similar to CHD2_HUMAN CHROMODOMAIN-HELICASE-DNA-BINDING PROTEIN 2 (CHD-2) [H.sapiens]
Hs.2794	DC12	DC12 protein
Hs.695	CSTB	cystatin B (stefin B)
Hs.66394	RNF4	ring finger protein 4
Hs.46638	C11orf8	chromosome 11 open reading frame 8
Hs.178738	CYP3A4	cytochrome P450, subfamily IIIA (niphedipine oxidase), polypeptide 4
Hs.171955	TROAP	trophinin associated protein (tastin)
Hs.2554	SIAT1	sialyltransferase 1 (beta-galactoside alpha-2,6-sialytransferase)
Hs.108969	PTD008	PTD008 protein
Hs.8878	KNSL1	kinesin-like 1
Hs.381152	GRB2	growth factor receptor-bound protein 2
Hs.79362	RBL2	retinoblastoma-like 2 (p130)
Hs.2490	CASP1	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)
Hs.376459	AA281945	Homo sapiens cDNA FLJ36300 fis, clone THYMU2004410, mRNA sequence
Hs.432811	DGUOK	deoxyguanosine kinase
Hs.3828	MVD	mevalonate (diphospho) decarboxylase
Hs.167927	ICA1	islet cell autoantigen 1, 69kDa
Hs.23964	SAP18	sin3-associated polypeptide, 18kDa
Hs.298184	KCNAB2	potassium voltage-gated channel, shaker-related subfamily, beta member 2
Hs.76507	PIG7	LPS-induced TNF-alpha factor
Hs.86948	SNRPD1	small nuclear ribonucleoprotein D1 polypeptide 16kDa
Hs.284259	DNAH9	dynein, axonemal, heavy polypeptide 9
Hs.54589	NCK1	NCK adaptor protein 1
Hs.1197	HSPE1	heat shock 10kDa protein 1 (chaperonin 10)
Hs.79350	RYK	RYK receptor-like tyrosine kinase
Hs.422340	SRI	sorcin
Hs.2507	HTR2B	5-hydroxytryptamine (serotonin) receptor 2B
Hs.125180	GHR	growth hormone receptor
Hs.81886	AUH	AU RNA binding protein/enoyl-Coenzyme A hydratase
Hs.152818	USP8	ubiquitin specific protease 8
Hs.163546	UBE2E1	ubiquitin-conjugating enzyme E2E 1 (UBC4/5 homolog, yeast)
Hs.355816	OS4	conserved gene amplified in osteosarcoma
Hs.687	CYP4B1	cytochrome P450, subfamily IVB, polypeptide 1
Hs.85258	CD8A	CD8 antigen, alpha polypeptide (p32)
Hs.17908	ORC1L	origin recognition complex, subunit 1-like (yeast)
Hs.296585	NOL5A	nucleolar protein 5A (56kDa with KKE/D repeat)
Hs.93002	UBE2C	ubiquitin-conjugating enzyme E2C
Hs.177486	APP	amyloid beta (A4) precursor protein (protease nexin-II, Alzheimer disease)
Hs.32970	SLAM	signaling lymphocytic activation molecule
Hs.2175	CSF3R	colony stimulating factor 3 receptor (granulocyte)
Hs.279061	CGI-150	CGI-150 protein
Hs.23103	BET1	BET1 homolog (S. cerevisiae)
Hs.73923	PNLIPRP1	pancreatic lipase-related protein 1
Hs.82318	WASF3	WAS protein family, member 3
Hs.250651	HF1	H factor 1 (complement)
Hs.433414	IFITM3	interferon induced transmembrane protein 3 (1-8U)

Hs.343575	ABI-2	abl-interactor 2
Hs.169895	UBE2L6	ubiquitin-conjugating enzyme E2L 6
Hs.1578	BIRC5	baculoviral IAP repeat-containing 5 (survivin)
Hs.75862	MADH4	MAD, mothers against decapentaplegic homolog 4 (Drosophila)
Hs.80986	ATP5G1	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 1
Hs.83656	ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta
Hs.60679	TAF9	TAF9 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 32kDa
Hs.105806	GNLY	granulysin
Hs.82985	COL5A2	collagen, type V, alpha 2
Hs.75811	ASAH1	N-acylsphingosine amidohydrolase (acid ceramidase) 1
Hs.80423	PBP	prostatic binding protein
Hs.44481	FOXF2	forkhead box F2
Hs.234279	MAPRE1	microtubule-associated protein, RP/EB family, member 1
Hs.125244	RAD51L3	RAD51-like 3 (S. cerevisiae)
Hs.250	XDH	xanthene dehydrogenase
Hs.45743	ADORA2B	adenosine A2b receptor
Hs.1987	CD28	CD28 antigen (Tp44)
Hs.196177	PHKG2	phosphorylase kinase, gamma 2 (testis)
Hs.355866	ZNF148	zinc finger protein 148 (pHZ-52)
Hs.166887	CPNE1	copine I
Hs.173737	RAC1	ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)
Hs.376966	AA035450	Homo sapiens mRNA; cDNA DKFZp313N1434 (from clone DKFZp313N1434), mRNA sequence
Hs.380768	AA459522	ESTs, Highly similar to A36670 cell division control protein CKS1 - human [H.sapiens]
Hs.74276	CLIC1	chloride intracellular channel 1
Hs.256747	SPAG8	sperm associated antigen 8
Hs.1162	HLA-DMB	major histocompatibility complex, class II, DM beta
Hs.184760	CBF2	CCAAT-box-binding transcription factor
Hs.432314	AA431967	Homo sapiens, clone IMAGE:4816693, mRNA, mRNA sequence
Hs.82128	TPBG	trophoblast glycoprotein
Hs.89575	CD79B	CD79B antigen (immunoglobulin-associated beta)
Hs.421241	ATP6V0E	ATPase, H+ transporting, lysosomal 9kDa, V0 subunit e
Hs.76095	IER3	immediate early response 3
Hs.38095	ABCA8	ATP-binding cassette, sub-family A (ABC1), member 8
Hs.89781	UBTF	upstream binding transcription factor, RNA polymerase I
Hs.8074	BAI3	brain-specific angiogenesis inhibitor 3
Hs.119598	RPL3	ribosomal protein L3
Hs.77273	ARHA	ras homolog gene family, member A
Hs.7019	SIPA1	signal-induced proliferation-associated gene 1
Hs.184222	DSCR1	Down syndrome critical region gene 1
Hs.83834	CYB5	cytochrome b-5
Hs.82911	PTP4A2	protein tyrosine phosphatase type IVA, member 2
Hs.146847	TANK	TRAF family member-associated NFKB activator
Hs.237356	CXCL12	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)
Hs.77558	HMGN3	high mobility group nucleosomal binding domain 3
Hs.2706	GPX4	glutathione peroxidase 4 (phospholipid hydroperoxidase)
Hs.82109	SDC1	syndecan 1
Hs.14286	FMO5	flavin containing monooxygenase 5
Hs.17483	CD4	CD4 antigen (p55)
Hs.169756	C1S	complement component 1, s subcomponent
Hs.4992	TSSC1	tumor suppressing subtransferable candidate 1
Hs.183684	EIF4G2	eukaryotic translation initiation factor 4 gamma, 2
Hs.2110	ZNF9	zinc finger protein 9 (a cellular retroviral nucleic acid binding protein)
Hs.411904	R55046	EST

Hs.432618	LOX	lysyl oxidase
Hs.80642	STAT4	signal transducer and activator of transcription 4
Hs.432580	DXF68S1E	DNA segment, numerous copies, expressed probes (GS1 gene)
Hs.1340	CLPS	colipase, pancreatic
Hs.125453	DARS	aspartyl-tRNA synthetase
Hs.159301	IL18R1	interleukin 18 receptor 1
Hs.98493	XRCC1	X-ray repair complementing defective repair in Chinese hamster cells 1
Hs.220255	POLR2J	polymerase (RNA) II (DNA directed) polypeptide J, 13.3kDa
Hs.90708	GZMA	granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)
Hs.154365	ELF1	E74-like factor 1 (ets domain transcription factor)
Hs.77899	TPM1	tropomyosin 1 (alpha)
Hs.147663	PTPN9	protein tyrosine phosphatase, non-receptor type 9
Hs.170019	RUNX3	runt-related transcription factor 3
Hs.44222	CGI-90	CGI-90 protein
Hs.78867	PTPRZ1	protein tyrosine phosphatase, receptor-type, Z polypeptide 1
Hs.171872	DDX8	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 8 (RNA helicase)
Hs.278270	TEBP	unactive progesterone receptor, 23 kD
Hs.155478	CCNT2	cyclin T2
Hs.256290	S100A11	S100 calcium binding protein A11 (calgizzarin)
Hs.184604	PPY	pancreatic polypeptide
Hs.421528	H94857	EST, Moderately similar to JC4392 RT14 protein - human [H.sapiens]
Hs.77202	PRKCB1	protein kinase C, beta 1
Hs.192570	FLJ22028	hypothetical protein FLJ22028
Hs.83758	CKS2	CDC28 protein kinase regulatory subunit 2
Hs.251064	HMGN1	high-mobility group nucleosome binding domain 1
Hs.336916	DAXX	death-associated protein 6
Hs.89519	KIAA1046	KIAA1046 protein
Hs.104925	ENC1	ectodermal-neural cortex (with BTB-like domain)
Hs.7655	U2AF65	U2 small nuclear ribonucleoprotein auxiliary factor (65kD)
Hs.31638	RSN	restin (Reed-Steinberg cell-expressed intermediate filament-associated protein)
Hs.23262	RNASE6	ribonuclease, RNase A family, k6
Hs.21365	NAP1L3	nucleosome assembly protein 1-like 3
Hs.173422	GBA2	glucosidase, beta (bile acid) 2
Hs.75428	SOD1	superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))
Hs.46405	POLR2F	polymerase (RNA) II (DNA directed) polypeptide F
Hs.82359	TNFRSF6	tumor necrosis factor receptor superfamily, member 6
Hs.180941	VPS41	vacuolar protein sorting 41 (yeast)
Hs.85050	PLN	phospholamban
Hs.5022	IPW	imprinted in Prader-Willi syndrome
Hs.334612	SNRPE	small nuclear ribonucleoprotein polypeptide E
Hs.2006	GSTM3	glutathione S-transferase M3 (brain)
Hs.107019	SPK	symplekin; Huntingtin interacting protein I
Hs.343586	ZFP36	zinc finger protein 36, C3H type, homolog (mouse)
Hs.24644	TAF4	TAF4 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 135kDa
Hs.155244	PRPF18	PRP18 pre-mRNA processing factor 18 homolog (yeast)
Hs.83636	ADRBK1	adrenergic, beta, receptor kinase 1
Hs.83958	TLE4	transducin-like enhancer of split 4 (E(sp1) homolog, Drosophila)
Hs.73956	NQO2	NAD(P)H dehydrogenase, quinone 2
Hs.367667	PMS2L8	postmeiotic segregation increased 2-like 8
Hs.28491	SAT	spermidine/spermine N1-acetyltransferase
Hs.87409	THBS1	thrombospondin 1
Hs.98008	GK2	glycerol kinase 2

Hs.172674	NFATC3	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3
Hs.119591	AP2S1	adaptor-related protein complex 2, sigma 1 subunit
Hs.250899	HSBP1	heat shock factor binding protein 1
Hs.326198	TCF4	transcription factor 4
Hs.172028	ADAM10	a disintegrin and metalloproteinase domain 10
Hs.85226	LIPA	lipase A, lysosomal acid, cholesterol esterase (Wolman disease)
Hs.19193	AA436990	ESTs, Weakly similar to WASL_RAT Neural Wiskott-Aldrich syndrome protein (N-WASP) [R.norvegicus]
Hs.81328	NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
Hs.433785	RNF5	ring finger protein 5
Hs.89455	OPRK1	opioid receptor, kappa 1
Hs.153261	IGHM	immunoglobulin heavy constant mu
Hs.77490	GSTT1	glutathione S-transferase theta 1
Hs.432976	AA629265	Homo sapiens cDNA FLJ34216 fis, clone FCBBF3022005, highly similar to OXYSTEROLS RECEPTOR LXR-BETA, mRNA sequence
Hs.76205	CYP11A	cytochrome P450, subfamily XIA (cholesterol side chain cleavage)
Hs.102950	IGLJ3	immunoglobulin lambda joining 3
Hs.433759	BCRP1	Breakpoint cluster region protein, uterine leiomyoma, 1; barrier to autointegration factor
Hs.93164	PCSK2	proprotein convertase subtilisin/kexin type 2
Hs.12272	BECN1	beclin 1 (coiled-coil, myosin-like BCL2 interacting protein)
Hs.103253	PLIN	perilipin
Hs.154207	CENPC1	centromere protein C 1
Hs.184326	CDC10	CDC10 cell division cycle 10 homolog (S. cerevisiae)
Hs.154655	MRPS31	mitochondrial ribosomal protein S31
Hs.173609	PSG4	pregnancy specific beta-1-glycoprotein 4
Hs.31210	BCL3	B-cell CLL/lymphoma 3
Hs.179665	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
Hs.74294	ALDH7A1	aldehyde dehydrogenase 7 family, member A1
Hs.272529	GPLD1	glycosylphosphatidylinositol specific phospholipase D1
Hs.24763	RANBP1	RAN binding protein 1
Hs.112049	SBF1	SET binding factor 1
Hs.727	INHBA	inhibin, beta A (activin A, activin AB alpha polypeptide)
Hs.118131	MTHFS	5,10-methenyltetrahydrofolate synthetase (5-formyltetrahydrofolate cyclo-ligase)
Hs.1652	CCR7	chemokine (C-C motif) receptor 7
Hs.343877	CGI-85	CGI-85 protein
Hs.2943	SRP19	signal recognition particle 19kDa
Hs.422414	H48122	ESTs, Highly similar to breast cancer 2, early onset [Homo sapiens] [H.sapiens]
Hs.74647	TRA@	T cell receptor alpha locus
Hs.73149	PAX8	paired box gene 8
Hs.50732	PRKAB2	protein kinase, AMP-activated, beta 2 non-catalytic subunit
Hs.48576	ERCC5	excision repair cross-complementing rodent repair deficiency, complementation group 5 (xeroderma pigmentosum, complementation group G (Cockayne syndrome))
Hs.3235	KRT4	keratin 4
Hs.228084	AA233643	ESTs, Weakly similar to 2109260A B cell growth factor [Homo sapiens] [H.sapiens]
Hs.279518	APLP2	amyloid beta (A4) precursor-like protein 2
Hs.27610	RI58	retinoic acid- and interferon-inducible protein (58kD)
Hs.76507	PIG7	LPS-induced TNF-alpha factor
Hs.74088	EGR3	early growth response 3
Hs.432942	AA411387	ESTs
Hs.349109	IGF2	insulin-like growth factor 2 (somatomedin A)
Hs.79630	CD79A	CD79A antigen (immunoglobulin-associated alpha)

Hs.7647	MAZ	MYC-associated zinc finger protein (purine-binding transcription factor)
Hs.169274	IFIT2	interferon-induced protein with tetratricopeptide repeats 2
Hs.2128	DUSP5	dual specificity phosphatase 5
Hs.70983	PARG1	PTPL1-associated RhoGAP 1
Hs.166468	PDCD5	programmed cell death 5
Hs.170285	NUP214	nucleoporin 214kDa
Hs.77515	ITPR3	inositol 1,4,5-triphosphate receptor, type 3
Hs.2164	PPBP	pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)
Hs.149098	SMTN	smoothelin
Hs.330994	RAB9A	RAB9A, member RAS oncogene family
Hs.6441	TIMP2	tissue inhibitor of metalloproteinase 2
Hs.1424	FMO1	flavin containing monooxygenase 1
Hs.380831	FOXO3A	forkhead box O3A
Hs.330310	AA496087	Unknown (protein for MGC:39264) [Homo sapiens], mRNA sequence
Hs.2815	POU6F1	POU domain, class 6, transcription factor 1
Hs.35140	STK4	serine/threonine kinase 4
Hs.301865	DCT	dopachrome tautomerase (dopachrome delta-isomerase, tyrosine-related protein 2)
Hs.8217	STAG2	stromal antigen 2
Hs.1376	HSD11B2	hydroxysteroid (11-beta) dehydrogenase 2
Hs.77273	ARHA	ras homolog gene family, member A
Hs.74637	TEGT	testis enhanced gene transcript (BAX inhibitor 1)
Hs.1252	APOH	apolipoprotein H (beta-2-glycoprotein I)
Hs.12230	SPP2	secreted phosphoprotein 2, 24kDa
Hs.83760	TNNI2	troponin I, skeletal, fast
Hs.31472	MAP3K7IP1	mitogen-activated protein kinase kinase kinase 7 interacting protein 1
Hs.296049	MFAP4	microfibrillar-associated protein 4
Hs.25537	CTF1	cardiotrophin 1
Hs.348397	PIGF	phosphatidylinositol glycan, class F
Hs.1516	IGFBP4	insulin-like growth factor binding protein 4
Hs.411850	AI583604	EST
Hs.89436	CDH17	cadherin 17, LI cadherin (liver-intestine)
Hs.422987	N66132	EST
Hs.1050	PSCD1	pleckstrin homology, Sec7 and coiled/coil domains 1(cytohesin 1)
Hs.59413	CTSL	cathepsin L
Hs.173854	PAXIP1L	PAX transcription activation domain interacting protein 1 like
Hs.75256	RGS1	regulator of G-protein signalling 1
Hs.269247	AA057328	ESTs
Hs.367689	TRIO	triple functional domain (PTPRF interacting)
Hs.180015	DDT	D-dopachrome tautomerase
Hs.100602	MADH7	MAD, mothers against decapentaplegic homolog 7 (Drosophila)
Hs.3280	CASP6	caspase 6, apoptosis-related cysteine protease
Hs.89862	TRADD	TNFRSF1A-associated via death domain
Hs.395771	CAT	catalase
Hs.89433	ABCC1	ATP-binding cassette, sub-family C (CFTR/MRP), member 1
Hs.351875	COX6C	cytochrome c oxidase subunit VIc
Hs.372732	EB-1	E2a-Pbx1-associated protein
Hs.380875	TEB4	similar to S. cerevisiae SSM4
Hs.296323	SGK	serum/glucocorticoid regulated kinase
Hs.423633	N95165	EST
Hs.155693	PTPN21	protein tyrosine phosphatase, non-receptor type 21
Hs.429	ATP5G3	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9) isoform 3
Hs.76293	TMSB10	thymosin, beta 10
Hs.77422	PLP2	proteolipid protein 2 (colonic epithelium-enriched)

Hs.77558	HMGN3	high mobility group nucleosomal binding domain 3
Hs.426123	AA088434	EST, Weakly similar to hypothetical protein MGC16040 [Homo sapiens] [H.sapiens]
Hs.16697	DR1	down-regulator of transcription 1, TBP-binding (negative cofactor 2)
Hs.75871	PRKCBP1	protein kinase C binding protein 1
Hs.380994	RBBP1	retinoblastoma binding protein 1
Hs.169993	DSPG3	dermatan sulfate proteoglycan 3
Hs.172471	KCNAB1	potassium voltage-gated channel, shaker-related subfamily, beta member 1
Hs.979	PDHB	pyruvate dehydrogenase (lipoamide) beta
Hs.333417	CAPZB	capping protein (actin filament) muscle Z-line, beta
Hs.10458	CCL16	chemokine (C-C motif) ligand 16
Hs.821	BGN	biglycan
Hs.169370	FYN	FYN oncogene related to SRC, FGR, YES
Hs.289056	H69833	ESTs, Moderately similar to KNG_HUMAN Kininogen precursor (Alpha-2-thiol proteinase inhibitor) [Contains: Bradykinin] [H.sapiens]
Hs.1430	F11	coagulation factor XI (plasma thromboplastin antecedent)
Hs.274122	EPB49	erythrocyte membrane protein band 4.9 (dematin)
Hs.432750	H62163	Homo sapiens cDNA FLJ34669 fis, clone LIVER2001051, mRNA sequence
Hs.76686	GPX1	glutathione peroxidase 1
Hs.417187	H06943	EST
Hs.75975	SRP9	signal recognition particle 9kDa
Hs.180612	PXMP3	peroxisomal membrane protein 3, 35kDa (Zellweger syndrome)
Hs.95577	CDK4	cyclin-dependent kinase 4
Hs.66052	CD38	CD38 antigen (p45)
Hs.84153	DCTN2	dynactin 2 (p50)
Hs.83429	TNFSF10	tumor necrosis factor (ligand) superfamily, member 10
Hs.214982	LAMC1	laminin, gamma 1 (formerly LAMB2)
Hs.423103	HPIP	HCF-1 beta-propeller interacting protein
Hs.151139	ELF4	E74-like factor 4 (ets domain transcription factor)
Hs.109752	RCL	putative c-Myc-responsive
Hs.55481	ZNF165	zinc finger protein 165
Hs.118442	CCNC	cyclin C
Hs.86122	GRCA	likely ortholog of mouse gene rich cluster, A gene
Hs.182231	TRH	thyrotropin-releasing hormone
Hs.433603	AA425973	ESTs, Moderately similar to KI67_HUMAN Antigen KI-67 [H.sapiens]
Hs.295923	SIAH1	seven in absentia homolog 1 (Drosophila)
Hs.29282	MAP3K3	mitogen-activated protein kinase kinase kinase 3
Hs.75716	SERPINB2	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 2
Hs.372664	OVCA2	candidate tumor suppressor OVCA2
Hs.356019	RPS5	ribosomal protein S5
Hs.75243	BRD2	bromodomain containing 2
Hs.165439	ASNA1	arsA arsenite transporter, ATP-binding, homolog 1 (bacterial)
Hs.3109	ARHGAP4	Rho GTPase activating protein 4
Hs.1915	FOLH1	folate hydrolase (prostate-specific membrane antigen) 1
Hs.82193	ESD	esterase D/formylglutathione hydrolase
Hs.337774	ARHGEF2	rho/rac guanine nucleotide exchange factor (GEF) 2
Hs.117176	PABPN1	poly(A) binding protein, nuclear 1
Hs.74561	A2M	alpha-2-macroglobulin
Hs.172609	NUCB1	nucleobindin 1
Hs.79015	MOX2	antigen identified by monoclonal antibody MRC OX-2
Hs.36566	LIMK1	LIM domain kinase 1
Hs.76780	PPP1R1A	protein phosphatase 1, regulatory (inhibitor) subunit 1A
Hs.241570	TNF	tumor necrosis factor (TNF superfamily, member 2)
Hs.14453	ICSBP1	interferon consensus sequence binding protein 1
Hs.15318	HAX1	HS1 binding protein

Hs.352382	SMG1	PI-3-kinase-related kinase SMG-1
Hs.362432	AI637939	ESTs
Hs.144567	AGXT	alanine-glyoxylate aminotransferase (oxalosis I; hyperoxaluria I; glycolicaciduria; serine-pyruvate aminotransferase)
Hs.178749	SSX3	synovial sarcoma, X breakpoint 3
Hs.343522	ATP2B4	ATPase, Ca++ transporting, plasma membrane 4
Hs.433703	W84789	Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 1695532, mRNA sequence
Hs.102867	SLC13A3	solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 3
Hs.89543	MCF2	MCF.2 cell line derived transforming sequence
Hs.66521	MJD	Machado-Joseph disease (spinocerebellar ataxia 3, olivopontocerebellar ataxia 3, autosomal dominant, ataxin 3)
Hs.423190	CRIP1	cysteine-rich protein 1 (intestinal)
Hs.379466	UBE2A	ubiquitin-conjugating enzyme E2A (RAD6 homolog)
Hs.82848	SELL	selectin L (lymphocyte adhesion molecule 1)
Hs.6574	DEAF1	deformed epidermal autoregulatory factor 1 (Drosophila)
Hs.6364	HTATIP	HIV-1 Tat interactive protein, 60kDa
Hs.105658	DFFA	DNA fragmentation factor, 45kDa, alpha polypeptide
Hs.100194	ALOX5AP	arachidonate 5-lipoxygenase-activating protein
Hs.429660	AA400474	ESTs, Highly similar to zona pellucida binding protein [Homo sapiens] [H.sapiens]
Hs.100932	ZNF354A	zinc finger protein 354A
Hs.16773	H54476	Homo sapiens clone TCCCIA00427 mRNA sequence
Hs.90318	MLC1SA	myosin light chain 1 slow a
Hs.154729	PDPK1	3-phosphoinositide dependent protein kinase-1
Hs.406277	SF3A1	splicing factor 3a, subunit 1, 120kDa
Hs.114408	TLR5	toll-like receptor 5
Hs.96247	TSNAX	translin-associated factor X
Hs.227789	MAPKAPK 3	mitogen-activated protein kinase-activated protein kinase 3
Hs.406521	AA862813	ESTs, Highly similar to 1501259A cytochrome c oxidase VIII [Homo sapiens] [H.sapiens]
Hs.80424	F13A1	coagulation factor XIII, A1 polypeptide
Hs.80691	CKMT2	creatine kinase, mitochondrial 2 (sarcomeric)
Hs.158295	MYL1	myosin, light polypeptide 1, alkali; skeletal, fast
Hs.46700	ING1	inhibitor of growth family, member 1
Hs.81988	DAB2	disabled homolog 2, mitogen-responsive phosphoprotein (Drosophila)
Hs.38084	SULT1C1	sulfotransferase family, cytosolic, 1C, member 1
Hs.25647	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog
Hs.374536	IVD	isovaleryl Coenzyme A dehydrogenase
Hs.399740	UPF3A	similar to yeast Upf3, variant A
Hs.8180	SDCBP	syndecan binding protein (syntenin)
Hs.819	HOXB7	homeo box B7
Hs.76452	CRP	C-reactive protein, pentraxin-related
Hs.388623	SFRS3	splicing factor, arginine/serine-rich 3
Hs.75615	APOC2	apolipoprotein C-II
Hs.96055	E2F1	E2F transcription factor 1
Hs.151899	SGCD	sarcoglycan, delta (35kDa dystrophin-associated glycoprotein)
Hs.80343	MMP15	matrix metalloproteinase 15 (membrane-inserted)
Hs.6727	HMGB1	high-mobility group box 1
Hs.119129	COL4A1	collagen, type IV, alpha 1
Hs.646	CPA3	carboxypeptidase A3 (mast cell)
Hs.274404	PLAT	plasminogen activator, tissue
Hs.378711	AA001443	Homo sapiens cDNA FLJ40855 fis, clone TRACH2016317, highly similar to HOMEOBOX PROTEIN MEIS1, mRNA sequence
Hs.332173	TLE2	transducin-like enhancer of split 2 (E(sp1) homolog, Drosophila)

Hs.250882	BDKRB2	bradykinin receptor B2
Hs.151738	MMP9	matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)
Hs.56845	GDI2	GDP dissociation inhibitor 2
Hs.159494	BTK	Bruton agammaglobulinemia tyrosine kinase
Hs.87539	ALDH3B2	aldehyde dehydrogenase 3 family, member B2
Hs.85266	ITGB4	integrin, beta 4
Hs.89791	WNT2	wingless-type MMTV integration site family member 2
Hs.239307	YARS	tyrosyl-tRNA synthetase
Hs.3745	MFGE8	milk fat globule-EGF factor 8 protein
Hs.147189	HYA22	HYA22 protein
Hs.89506	PAX6	paired box gene 6 (aniridia, keratitis)
Hs.83916	NDUFA5	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13kDa
Hs.315177	IFRD2	interferon-related developmental regulator 2
Hs.81874	MGST2	microsomal glutathione S-transferase 2
Hs.12477	SNAP91	synaptosomal-associated protein, 91kDa homolog (mouse)
Hs.75760	SCP2	sterol carrier protein 2
Hs.374990	CD34	CD34 antigen
Hs.150595	CYP26A1	cytochrome P450, subfamily XXVIA, polypeptide 1
Hs.102171	ISLR	immunoglobulin superfamily containing leucine-rich repeat
Hs.80205	PIM2	pim-2 oncogene
Hs.77432	EGFR	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
Hs.131255	UQCRB	ubiquinol-cytochrome c reductase binding protein
Hs.102122	IGFBP1	insulin-like growth factor binding protein 1
Hs.181289	ELA3A	elastase 3A, pancreatic (protease E)
Hs.90875	RABIF	RAB interacting factor
Hs.177582	SFTPA2	surfactant, pulmonary-associated protein A2
Hs.155584	KIAA0121	KIAA0121 gene product
Hs.151413	GMFB	glia maturation factor, beta
Hs.89485	CA4	carbonic anhydrase IV
Hs.6361	MAP2K1IP 1	mitogen-activated protein kinase kinase 1 interacting protein 1
Hs.348935	IGLL1	immunoglobulin lambda-like polypeptide 1
Hs.7306	SFRP1	secreted frizzled-related protein 1
Hs.54460	CCL11	chemokine (C-C motif) ligand 11
Hs.154718	TPD52L2	tumor protein D52-like 2
Hs.336916	DAXX	death-associated protein 6
Hs.505	ISL1	ISL1 transcription factor, LIM/homeodomain, (islet-1)
Hs.2384	TPD52	tumor protein D52
Hs.43697	ETV5	ets variant gene 5 (ets-related molecule)
Hs.127799	BIRC3	baculoviral IAP repeat-containing 3
Hs.281960	OAZ1	ornithine decarboxylase antizyme 1
Hs.352537	AA190993	Homo sapiens cDNA FLJ31066 fis, clone HSYRA2001153, mRNA sequence
Hs.179982	TP53BPL	tumor protein p53-binding protein
Hs.79516	BASP1	brain abundant, membrane attached signal protein 1
Hs.349845	AA479102	Homo sapiens cDNA FLJ32993 fis, clone THYMU1000103, weakly similar to PROTEIN KINASE C, BETA-I TYPE (EC 2.7.1.-), mRNA sequence
Hs.11171	APG5L	APG5 autophagy 5-like (S. cerevisiae)
Hs.433291	ARD1	ARD1 homolog, N-acetyltransferase (S. cerevisiae)
Hs.2962	S100P	S100 calcium binding protein P
Hs.267445	MAPK8	mitogen-activated protein kinase 8
Hs.91096	TRIM31	tripartite motif-containing 31
Hs.85112	IGF1	insulin-like growth factor 1 (somatomedin C)
Hs.1298	MME	membrane metallo-endopeptidase (neutral endopeptidase, enkephalinase, CALLA, CD10)

Hs.169248	CYCS	cytochrome c, somatic
Hs.90408	NEO1	neogenin homolog 1 (chicken)
Hs.179665	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
Hs.153932	PTPN3	protein tyrosine phosphatase, non-receptor type 3
Hs.2891	PRKCM	protein kinase C, mu
Hs.17287	KCNJ15	potassium inwardly-rectifying channel, subfamily J, member 15
Hs.78877	ITPKB	inositol 1,4,5-trisphosphate 3-kinase B
Hs.99171	NTF3	neurotrophin 3
Hs.236030	SMARCC2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2
Hs.41693	DNAJB4	DnaJ (Hsp40) homolog, subfamily B, member 4
Hs.380778	MT1L	metallothionein 1L
Hs.76289	BLVRB	biliverdin reductase B (flavin reductase (NADPH))
Hs.433205	AA872383	Similar to RNA helicase-related protein [Homo sapiens], mRNA sequence
Hs.183738	FARP1	FERM, RhoGEF (ARHGEF) and pleckstrin domain protein 1 (chondrocyte-derived)
Hs.333497	CYP2D6	cytochrome P450, subfamily IID (debrisoquine, sparteine, etc., -metabolizing), polypeptide 6
Hs.83919	GCS1	glucosidase I
Hs.158200	EGFL4	EGF-like-domain, multiple 4
Hs.5344	AP1G1	adaptor-related protein complex 1, gamma 1 subunit
Hs.8037	TM4SF9	tetraspan 5
Hs.118442	CCNC	cyclin C
Hs.78183	AKR1C3	aldo-keto reductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, type II)
Hs.433779	EEF1E1	eukaryotic translation elongation factor 1 epsilon 1
Hs.93597	CDK5R1	cyclin-dependent kinase 5, regulatory subunit 1 (p35)
Hs.100724	PPARG	peroxisome proliferative activated receptor, gamma
Hs.351476	AA497038	Homo sapiens cDNA FLJ33111 fis, clone TRACH2001085, mRNA sequence
Hs.80988	COL6A3	collagen, type VI, alpha 3
Hs.79026	MLF2	myeloid leukemia factor 2
Hs.432607	PSMB2	proteasome (prosome, macropain) subunit, beta type, 2
Hs.80658	UCP2	uncoupling protein 2 (mitochondrial, proton carrier)
Hs.11958	RODH	3-hydroxysteroid epimerase
Hs.194035	C14orf92	chromosome 14 open reading frame 92
Hs.3080	MAPK7	mitogen-activated protein kinase 7
Hs.27424	DDX11	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL1-like helicase homolog, S. cerevisiae)
Hs.75721	PFN1	profilin 1
Hs.374518	FTH1	ferritin, heavy polypeptide 1
Hs.20478	CLN2	ceroid-lipofuscinosis, neuronal 2, late infantile (Jansky-Bielschowsky disease)
Hs.283738	CSNK1A1	casein kinase 1, alpha 1
Hs.77171	MCM5	MCM5 minichromosome maintenance deficient 5, cell division cycle 46 (S. cerevisiae)
Hs.51233	TNFRSF10B	tumor necrosis factor receptor superfamily, member 10b
Hs.16426	PODXL	podocalyxin-like
Hs.87149	ITGB3	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
Hs.184014	RPL31	ribosomal protein L31
Hs.3254	MRPL23	mitochondrial ribosomal protein L23
Hs.50966	CPS1	carbamoyl-phosphate synthetase 1, mitochondrial
Hs.263671	RDX	radixin
Hs.284279	HMOX2	heme oxygenase (decycling) 2
Hs.26550	RXRG	retinoid X receptor, gamma
Hs.5299	ALDH5A1	aldehyde dehydrogenase 5 family, member A1 (succinate-

		semialdehyde dehydrogenase)
Hs.88411	NCR3	natural cytotoxicity triggering receptor 3
Hs.301819	ZNF146	zinc finger protein 146
Hs.82890	DAD1	defender against cell death 1
Hs.283844	BA108L7.2	similar to rat tricarboxylate carrier-like protein
Hs.182255	NHP2L1	NHP2 non-histone chromosome protein 2-like 1 (S. cerevisiae)
Hs.348024	RALB	v-ral simian leukemia viral oncogene homolog B (ras related; GTP binding protein)
Hs.50651	JAK1	Janus kinase 1 (a protein tyrosine kinase)
Hs.184916	CLTCL1	clathrin, heavy polypeptide-like 1
Hs.78353	SRPK2	SFRS protein kinase 2
Hs.7644	H1F2	H1 histone family, member 2
Hs.234734	LYZ	lysozyme (renal amyloidosis)
Hs.143482	PPID	peptidylprolyl isomerase D (cyclophilin D)
Hs.1608	RPA3	replication protein A3, 14kDa
Hs.1742	IQGAP1	IQ motif containing GTPase activating protein 1
Hs.155356	MGC2840	hypothetical protein MGC2840 similar to a putative glucosyltransferase
Hs.283565	FOSL1	FOS-like antigen 1
Hs.71346	NEF3	neurofilament 3 (150kDa medium)
Hs.159581	MMP17	matrix metalloproteinase 17 (membrane-inserted)
Hs.394	ADM	adrenomedullin
Hs.32935	BRF1	BRF1 homolog, subunit of RNA polymerase III transcription initiation factor IIIB (S. cerevisiae)
Hs.104636	TULP2	tubby like protein 2
Hs.79194	CREB1	cAMP responsive element binding protein 1
Hs.16530	CCL18	chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)
Hs.198308	WRB	tryptophan rich basic protein
Hs.75302	MTM1	myotubular myopathy 1
Hs.118021	ABR	active BCR-related gene
Hs.78436	EPHB1	EphB1
Hs.1657	ESR1	estrogen receptor 1
Hs.79300	UBE2V2	ubiquitin-conjugating enzyme E2 variant 2
Hs.377067	MAP3K13	mitogen-activated protein kinase kinase kinase 13
Hs.17820	ROCK1	Rho-associated, coiled-coil containing protein kinase 1
Hs.151734	NUTF2	nuclear transport factor 2
Hs.69285	NRP1	neuropilin 1
Hs.118397	AEBP1	AE binding protein 1
Hs.1227	ALAD	aminolevulinate, delta-, dehydratase
Hs.380923	MEIS3	Meis1, myeloid ecotropic viral integration site 1 homolog 3 (mouse)
Hs.171909	U2AF1RS2	U2 small nuclear ribonucleoprotein auxiliary factor, small subunit 2
Hs.20938	RBMS2	RNA binding motif, single stranded interacting protein 2
Hs.36	LTA	lymphotoxin alpha (TNF superfamily, member 1)
Hs.107164	SPTBN1	spectrin, beta, non-erythrocytic 1
Hs.6061	PRKAB1	protein kinase, AMP-activated, beta 1 non-catalytic subunit
Hs.420106	ENDOG	endonuclease G
Hs.174185	ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)
Hs.89656	TEC	tec protein tyrosine kinase
Hs.332053	SAA1	serum amyloid A1
Hs.81665	KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
Hs.239500	MGC13114	hypothetical protein MGC13114
Hs.514	CCNH	cyclin H
Hs.75082	ARHG	ras homolog gene family, member G (rho G)
Hs.180930	BTAF1	BTAF1 RNA polymerase II, B-TFIID transcription factor-associated, 170kDa (Mot1 homolog, S. cerevisiae)
Hs.36102	H72723	ESTs, Highly similar to MT1B_HUMAN METALLOTHIONEIN-IB (MT-1B) [H.sapiens]

Hs.158174	ZNF184	zinc finger protein 184 (Kruppel-like)
Hs.166011	CTNND1	catenin (cadherin-associated protein), delta 1
Hs.279903	RHEB2	Ras homolog enriched in brain 2
Hs.433410	MNAT1	menage a trois 1 (CAK assembly factor)
Hs.174142	CSF1R	colony stimulating factor 1 receptor, formerly McDonough feline sarcoma viral (v-fms) oncogene homolog
Hs.82887	PPP1R11	protein phosphatase 1, regulatory (inhibitor) subunit 11
Hs.154695	PMM2	phosphomannomutase 2
Hs.10712	PTEN	phosphatase and tensin homolog (mutated in multiple advanced cancers 1)
Hs.3041	UNG2	uracil-DNA glycosylase 2
Hs.5422	GPM6B	glycoprotein M6B
Hs.195464	FLNA	filamin A, alpha (actin binding protein 280)
Hs.171014	VGF	VGF nerve growth factor inducible
Hs.349530	YWHAH	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide
Hs.902	NF2	neurofibromin 2 (bilateral acoustic neuroma)
Hs.84113	CDKN3	cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)
Hs.154095	JDP2	jun dimerization protein 2
Hs.432953	AI697503	ESTs
Hs.3066	GZMK	granzyme K (serine protease, granzyme 3; tryptase II)
Hs.425168	AI566155	Homo sapiens cDNA FLJ11441 fis, clone HEMBA1001323, mRNA sequence
Hs.23960	CCNB1	cyclin B1
Hs.61152	EXTL2	exostoses (multiple)-like 2
Hs.79933	CCNI	cyclin I
Hs.89603	MUC1	mucin 1, transmembrane
Hs.59242	FURIN	furin (paired basic amino acid cleaving enzyme)
Hs.301394	MGC3101	hypothetical protein MGC3101
Hs.289082	GM2A	GM2 ganglioside activator protein
Hs.166891	RFX5	regulatory factor X, 5 (influences HLA class II expression)
Hs.301404	RBM3	RNA binding motif protein 3
Hs.424414	MSX1	msh homeo box homolog 1 (Drosophila)
Hs.924	CRYM	crystallin, mu
Hs.279910	ATOX1	ATX1 antioxidant protein 1 homolog (yeast)
Hs.76888	INA	internexin neuronal intermediate filament protein, alpha
Hs.89414	CXCR4	chemokine (C-X-C motif) receptor 4
Hs.251850	PSG5	pregnancy specific beta-1-glycoprotein 5
Hs.258850	KLRC3	killer cell lectin-like receptor subfamily C, member 3
Hs.82037	TAF12	TAF12 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 20kDa
Hs.25723	SSSCA1	Sjogren's syndrome/scleroderma autoantigen 1
Hs.15164	C1D	nuclear DNA-binding protein
Hs.75643	NFE2	nuclear factor (erythroid-derived 2), 45kDa
Hs.77978	DKFZP761I2123	KIAA1886 protein
Hs.157441	SPI1	spleen focus forming virus (SFFV) proviral integration oncogene spi1
Hs.29499	TLR3	toll-like receptor 3
Hs.153498	C18orf1	chromosome 18 open reading frame 1
Hs.247879	AA161390	Homo sapiens cDNA FLJ35073 fis, clone PLACE6000630, mRNA sequence
Hs.431043	PKNOX1	PBX/knotted 1 homeobox 1
Hs.75596	IL2RB	interleukin 2 receptor, beta
Hs.15384	AP1GBP1	AP1 gamma subunit binding protein 1
Hs.118638	NME1	non-metastatic cells 1, protein (NM23A) expressed in
Hs.182741	TIAL1	TIA1 cytotoxic granule-associated RNA binding protein-like 1
Hs.96063	IRS1	insulin receptor substrate 1

Hs.78885	BTD	biotinidase
Hs.74502	CTRB1	chymotrypsinogen B1
Hs.19192	CDK2	cyclin-dependent kinase 2
Hs.91161	PFDN4	prefoldin 4
Hs.2488	LCP2	lymphocyte cytosolic protein 2 (SH2 domain containing leukocyte protein of 76kDa)
Hs.74598	POLD2	polymerase (DNA directed), delta 2, regulatory subunit 50kDa
Hs.390278	PSG3	pregnancy specific beta-1-glycoprotein 3
Hs.326248	PDCD4	programmed cell death 4 (neoplastic transformation inhibitor)
Hs.1313	TNFSF8	tumor necrosis factor (ligand) superfamily, member 8
Hs.91299	GNB2	guanine nucleotide binding protein (G protein), beta polypeptide 2
Data not found	AA279677	
Hs.5085	DPM1	dolichyl-phosphate mannosyltransferase polypeptide 1, catalytic subunit
Hs.46423	H4FG	H4 histone family, member G
Hs.1030	RIN1	Ras and Rab interactor 1
Hs.373648	ZNF137	zinc finger protein 137 (clone pHZ-30)
Hs.2714	FOXG1B	forkhead box G1B
Hs.80828	KRT1	keratin 1 (epidermolytic hyperkeratosis)
Hs.425457	AA045180	EST
Hs.369508	PSPHL	phosphoserine phosphatase-like
Hs.155924	CREM	cAMP responsive element modulator
Hs.371342	AA256507	ESTs, Highly similar to uronyl-2-sulfotransferase; dermatan/chondroitin sulfate 2-sulfotransferase; uronyl 2-sulfotransferase [Homo sapiens] [H.sapiens]
Hs.1279	C1R	complement component 1, r subcomponent
Hs.95577	CDK4	cyclin-dependent kinase 4
Hs.139226	RFC2	replication factor C (activator 1) 2, 40kDa
Hs.24752	SSH3BP1	spectrin SH3 domain binding protein 1
Hs.197345	G22P1	thyroid autoantigen 70kDa (Ku antigen)
Hs.110675	APOC4	apolipoprotein C-IV
Hs.380716	AA634103	Human promyelocytic leukemia cell mRNA, clones pHH58 and pHH81, mRNA sequence
Hs.92858	GUCA1A	guanylate cyclase activator 1A (retina)
Hs.73073	ANKRD7	ankyrin repeat domain 7
Hs.422892	N57964	EST
Hs.87268	ANXA8	annexin A8
Hs.91065	RBBP6	retinoblastoma binding protein 6
Hs.37936	SUV39H1	suppressor of variegation 3-9 homolog 1 (Drosophila)
Hs.80887	LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog
Hs.76894	DCTD	dCMP deaminase
Hs.9731	NFKBIB	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta
Hs.167503	STAT5A	signal transducer and activator of transcription 5A
Hs.91566	PL6	PL6 protein
Hs.106070	CDKN1C	cyclin-dependent kinase inhibitor 1C (p57, Kip2)
Hs.241561	PRSS2	protease, serine, 2 (trypsin 2)
Hs.250897	TFG	TRK-fused gene
Hs.51120	CAMP	cathelicidin antimicrobial peptide
Hs.122566	CRYGA	crystallin, gamma A
Hs.429847	AA454702	EST, Weakly similar to RET3_HUMAN Retinoic acid-binding protein I, cellular (CRABP-I) [H.sapiens]
Hs.313869	AW473840	ESTs
Hs.82689	TRA1	tumor rejection antigen (gp96) 1
Hs.29352	TNFAIP6	tumor necrosis factor, alpha-induced protein 6
Hs.226307	APOBEC3B	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B

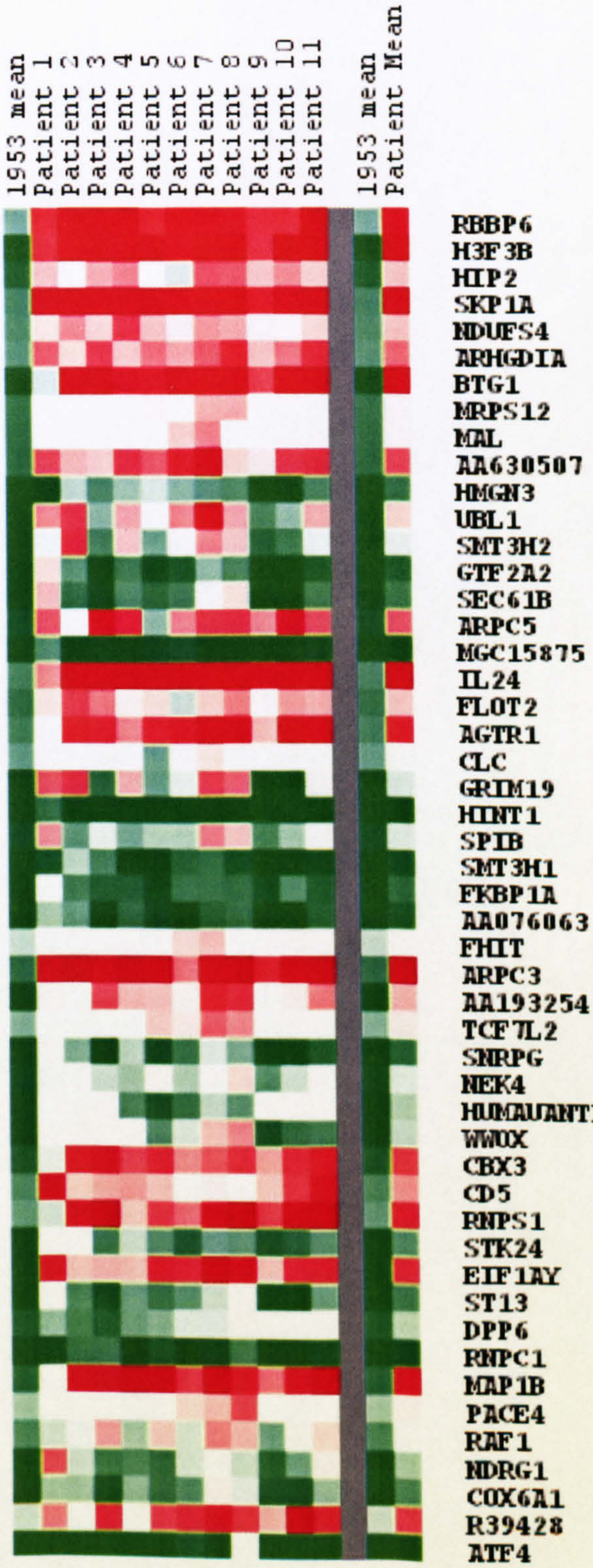
Hs.92282	PITX2	paired-like homeodomain transcription factor 2
Hs.100000	S100A8	S100 calcium binding protein A8 (calgranulin A)
Hs.169300	TGFB2	transforming growth factor, beta 2
Hs.100322	CA6	carbonic anhydrase VI
Hs.2316	SOX9	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)
Hs.85539	ATP5I	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit e
Hs.433168	S100A3	S100 calcium binding protein A3
Hs.79337	PASK	PAS domain containing serine/threonine kinase
Hs.123078	TSHR	thyroid stimulating hormone receptor
Hs.78979	GLG1	golgi apparatus protein 1
Hs.56937	ST14	suppression of tumorigenicity 14 (colon carcinoma, matriptase, epithin)
Hs.1042	SSA1	Sjogren syndrome antigen A1 (52kDa, ribonucleoprotein autoantigen SS-A/Ro)
Hs.143288	MGC11271	hypothetical protein MGC11271
Hs.157	KEL	Kell blood group
Hs.50924	GATA6	GATA binding protein 6
Hs.180919	ID2	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
Hs.4	ADH1B	alcohol dehydrogenase IB (class I), beta polypeptide
Hs.129804	AA453993	EST, Weakly similar to hypothetical protein DKFZp434F1726 [Homo sapiens] [H.sapiens]
Hs.149846	ITGB5	integrin, beta 5
Hs.192803	XPA	xeroderma pigmentosum, complementation group A
Hs.166120	IRF7	interferon regulatory factor 7
Hs.155090	GNB5	guanine nucleotide binding protein (G protein), beta 5
Hs.75393	ACP1	acid phosphatase 1, soluble
Hs.334790	FLJ14675	hypothetical protein FLJ14675
Hs.424362	DCTN6	likely ortholog of mouse dynactin 6
Hs.183601	RGS16	regulator of G-protein signalling 16
Hs.241570	TNF	tumor necrosis factor (TNF superfamily, member 2)
Hs.150917	CTNNA2	catenin (cadherin-associated protein), alpha 2
Hs.425103	AA017535	EST
Hs.23994	ACVR2B	activin A receptor, type IIB
Hs.9994	LIPC	lipase, hepatic
Hs.89626	PTHLH	parathyroid hormone-like hormone
Hs.387381	H15703	ESTs
Hs.116784	TRIP4	thyroid hormone receptor interactor 4
Hs.1906	PRLR	prolactin receptor
Hs.153837	MNDA	myeloid cell nuclear differentiation antigen
Hs.161362	PIN1	protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting 1
Hs.75367	SLA	Src-like-adaptor
Hs.3709	QP-C	low molecular mass ubiquinone-binding protein (9.5kD)
Hs.151641	GARP	glycoprotein A repetitions predominant
Hs.119014	ZNF175	zinc finger protein 175
Hs.54481	LRP8	low density lipoprotein receptor-related protein 8, apolipoprotein e receptor
Hs.279943	LMCD1	LIM and cysteine-rich domains 1
Hs.25051	PKP2	plakophilin 2
Hs.923	SSBP1	single-stranded DNA binding protein
Hs.24422	RFXAP	regulatory factor X-associated protein
Hs.24194	FOLR2	folate receptor 2 (fetal)
Hs.193788	NOS2A	nitric oxide synthase 2A (inducible, hepatocytes)
Hs.288658	ZNF35	zinc finger protein 35 (clone HF.10)
Hs.180533	MAP2K3	mitogen-activated protein kinase kinase 3
Hs.159608	ALDH3A2	aldehyde dehydrogenase 3 family, member A2
Hs.176977	OLIG2	oligodendrocyte lineage transcription factor 2
Hs.347979	R74169	ESTs, Highly similar to A43542 lymphocyte-specific protein 1 -

		human [H.sapiens]
Hs.33021	NOVA2	neuro-oncological ventral antigen 2
Hs.153179	RPLP2	ribosomal protein, large P2
Hs.99872	FALZ	fetal Alzheimer antigen
Hs.53985	GP2	glycoprotein 2 (zymogen granule membrane)
Hs.418506	INSL4	insulin-like 4 (placenta)
Hs.406013	KRT18	keratin 18
Hs.1408	EDN3	endothelin 3
Hs.166563	RFC1	replication factor C (activator 1) 1, 145kDa
Hs.75823	AF1Q	ALL1-fused gene from chromosome 1q
Hs.788	AKAP12	A kinase (PRKA) anchor protein (gravin) 12
Hs.2291	TG737	Probe hTg737 (polycystic kidney disease, autosomal recessive, in)
Hs.202833	HMOX1	heme oxygenase (decycling) 1
Hs.9999	EMP3	epithelial membrane protein 3
Hs.83920	PAM	peptidylglycine alpha-amidating monooxygenase
Hs.139851	CAV2	caveolin 2
Hs.429720	T88890	Homo sapiens cDNA FLJ36637 fis, clone TRACH2018914, highly similar to ZINC FINGER PROTEIN 169, mRNA sequence
Hs.31339	FGF11	fibroblast growth factor 11
Hs.20019	HFE	hemochromatosis
Hs.150403	DDC	dopa decarboxylase (aromatic L-amino acid decarboxylase)
Hs.282975	CES2	carboxylesterase 2 (intestine, liver)
Hs.82963	GNRH1	gonadotropin-releasing hormone 1 (leutinizing-releasing hormone)
Hs.75334	EXT2	exostoses (multiple) 2
Hs.112360	PROML1	prominin-like 1 (mouse)
Hs.82163	MAOB	monoamine oxidase B
Hs.119007	RAB4A	RAB4A, member RAS oncogene family
Hs.416967	R80979	Homo sapiens cDNA FLJ33295 fis, clone BNGH42000815, highly similar to Human SH3 domain-containing proline-rich kinase (sprk) mRNA, mRNA sequence
Hs.28988	GLRX	glutaredoxin (thioltransferase)
Hs.709	DCK	deoxycytidine kinase
Hs.9615	MYL9	myosin, light polypeptide 9, regulatory
Hs.89546	SELE	selectin E (endothelial adhesion molecule 1)
Hs.433737	RAN	RAN, member RAS oncogene family
Hs.166204	PHF1	PHD finger protein 1
Hs.26045	PTPRA	protein tyrosine phosphatase, receptor type, A
Hs.74615	PDGFRA	platelet-derived growth factor receptor, alpha polypeptide
Hs.172979	ZNF177	zinc finger protein 177
Hs.272429	CASR	calcium-sensing receptor (hypocalciuric hypercalcemia 1, severe neonatal hyperparathyroidism)
Hs.78913	CX3CR1	chemokine (C-X3-C motif) receptor 1
Hs.153768	U3-55K	U3 snoRNP-associated 55-kDa protein
Hs.381034	PPP1R2	protein phosphatase 1, regulatory (inhibitor) subunit 2
Hs.432667	AA169798	ESTs, Highly similar to A56716 aromatic ester hydrolase (EC 3.1.1.-) - human [H.sapiens]
Hs.147049	CUTL1	cut-like 1, CCAAT displacement protein (Drosophila)
Hs.424966	PIR	Pirin
Hs.2719	WFDC2	WAP four-disulfide core domain 2
Hs.421376	H85749	ESTs, Highly similar to deleted in split-hand/split-foot 1 region [Homo sapiens] [H.sapiens]
Hs.239	FOXM1	forkhead box M1
Hs.76289	BLVRB	biliverdin reductase B (flavin reductase (NADPH))
Hs.29287	RBBP8	retinoblastoma binding protein 8
Hs.129844	TNFRSF10D	tumor necrosis factor receptor superfamily, member 10d, decoy with truncated death domain
Hs.182280	MEF2A	MADS box transcription enhancer factor 2, polypeptide A (myocyte enhancer factor 2A)

Hs.146409	CDC42	cell division cycle 42 (GTP binding protein, 25kDa)
Hs.92458	GPR19	G protein-coupled receptor 19
Hs.171945	PLA2R1	phospholipase A2 receptor 1, 180kDa
Hs.79069	AA489647	Homo sapiens mRNA; cDNA DKFZp434B142 (from clone DKFZp434B142), mRNA sequence
Hs.380935	HSF1	heat shock transcription factor 1
Hs.381218	CYB5-M	cytochrome b5 outer mitochondrial membrane precursor
Hs.8024	IK	IK cytokine, down-regulator of HLA II
Hs.6129	ABCB9	ATP-binding cassette, sub-family B (MDR/TAP), member 9
Hs.75305	AIP	aryl hydrocarbon receptor interacting protein
Hs.2877	CDH3	cadherin 3, type 1, P-cadherin (placental)
Hs.2777	ITIH1	inter-alpha (globulin) inhibitor, H1 polypeptide
Hs.405200	R02373	EST
Hs.105938	LTF	lactotransferrin
Hs.352	FOLR3	folate receptor 3 (gamma)
Hs.82283	MTR	5-methyltetrahydrofolate-homocysteine methyltransferase
Hs.151988	MAP3K5	mitogen-activated protein kinase kinase kinase 5
Hs.171862	GBP2	guanylate binding protein 2, interferon-inducible
Hs.172323	CYP3A7	cytochrome P450, subfamily IIIA, polypeptide 7
Hs.421514	N51030	ESTs
Hs.5464	SMAP	skeletal muscle abundant protein
Hs.9018	EXTL3	exostoses (multiple)-like 3
Hs.432664	AA702663	Myosin, mRNA sequence
Hs.75746	ALDH1A3	aldehyde dehydrogenase 1 family, member A3
Hs.83727	CPSF1	cleavage and polyadenylation specific factor 1, 160kDa
Hs.406278	PLGL	plasminogen-like
Hs.839	IGFALS	insulin-like growth factor binding protein, acid labile subunit
Hs.279862	BCCIP	BRCA2 and CDKN1A interacting protein
Hs.10712	PTEN	phosphatase and tensin homolog (mutated in multiple advanced cancers 1)
Hs.176663	FCGR3A	Fc fragment of IgG, low affinity IIIa, receptor for (CD16)
Hs.303649	CCL2	chemokine (C-C motif) ligand 2
Hs.427586	H90815	ESTs, Highly similar to CBG_HUMAN Corticosteroid-binding globulin precursor (CBG) (Transcortin) [H.sapiens]
Hs.278275	TPSG1	tryptase gamma 1
Hs.279032	HUMGT198A	GT198, complete ORF
Hs.283476	PTE1	peroxisomal acyl-CoA thioesterase
Hs.1395	EGR2	early growth response 2 (Krox-20 homolog, Drosophila)
Hs.83341	AXL	AXL receptor tyrosine kinase
Hs.79241	BCL2	B-cell CLL/lymphoma 2
Hs.300772	TPM2	tropomyosin 2 (beta)
Hs.13137	UVRAG	UV radiation resistance associated gene
Hs.278388	ORM2	orosomucoid 2
Hs.12503	IL15RA	interleukin 15 receptor, alpha
Hs.380388	MC1R	melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor)
Hs.75474	NPHP1	nephronophthisis 1 (juvenile)
Hs.5591	MKNK1	MAP kinase-interacting serine/threonine kinase 1
Hs.11101	DLG3	discs, large (Drosophila) homolog 3 (neuroendocrine-dlg)
Hs.840	INDO	indoleamine-pyrrole 2,3 dioxygenase
Hs.153618	HCGVIII-1	HCGVIII-1 protein
Hs.79732	FBLN1	fibulin 1
Hs.83164	COL15A1	collagen, type XV, alpha 1
Hs.315463	IL24	interleukin 24
Hs.37616	MGC14480	hypothetical protein MGC14480
Hs.348401	LCAT	lecithin-cholesterol acyltransferase
Hs.68879	BMP4	bone morphogenetic protein 4

Hs.166846	POLE	polymerase (DNA directed), epsilon
Hs.1897	POMC	proopiomelanocortin (adrenocorticotropin/ beta-lipotropin/ alpha-melanocyte stimulating hormone/ beta-melanocyte stimulating hormone/ beta-endorphin)
Hs.79876	STS	steroid sulfatase (microsomal), arylsulfatase C, isozyme S
Hs.1209	ACADL	acyl-Coenzyme A dehydrogenase, long chain
Hs.75562	DDR1	discoidin domain receptor family, member 1
Hs.16362	P2RY6	pyrimidinergic receptor P2Y, G-protein coupled, 6
Hs.94865	TEAD4	TEA domain family member 4
Hs.155079	PPP2R5A	protein phosphatase 2, regulatory subunit B (B56), alpha isoform
Hs.388927	AA491227	ESTs, Weakly similar to A56419 kappaE3' enhancer-binding protein NF-E1 - human [H.sapiens]
Hs.59106	CGR19	cell growth regulatory with ring finger domain
Hs.433898	GNG10	guanine nucleotide binding protein (G protein), gamma 10

1.2 Genes with significantly increased polysome association in B-CLL cells when compared to GM1953 cells. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated.

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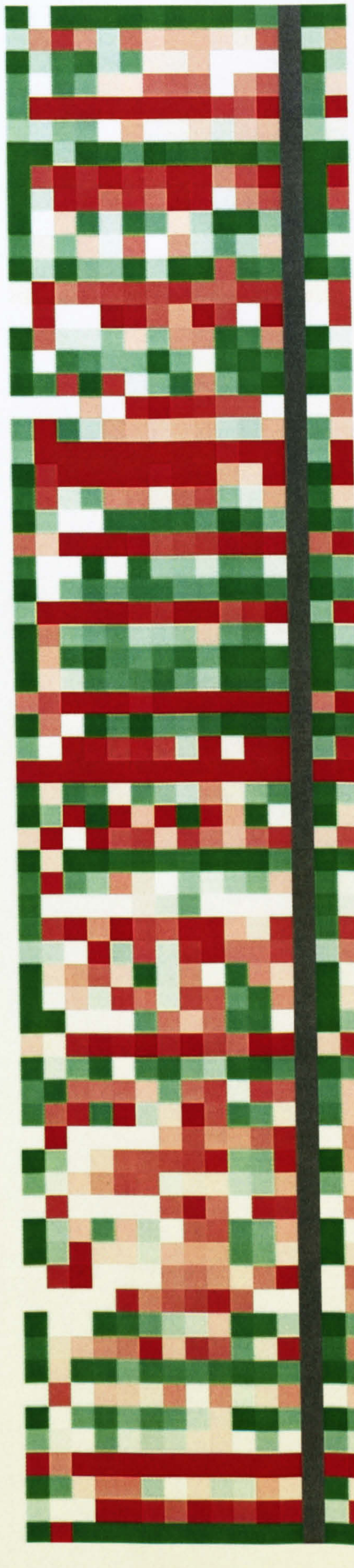
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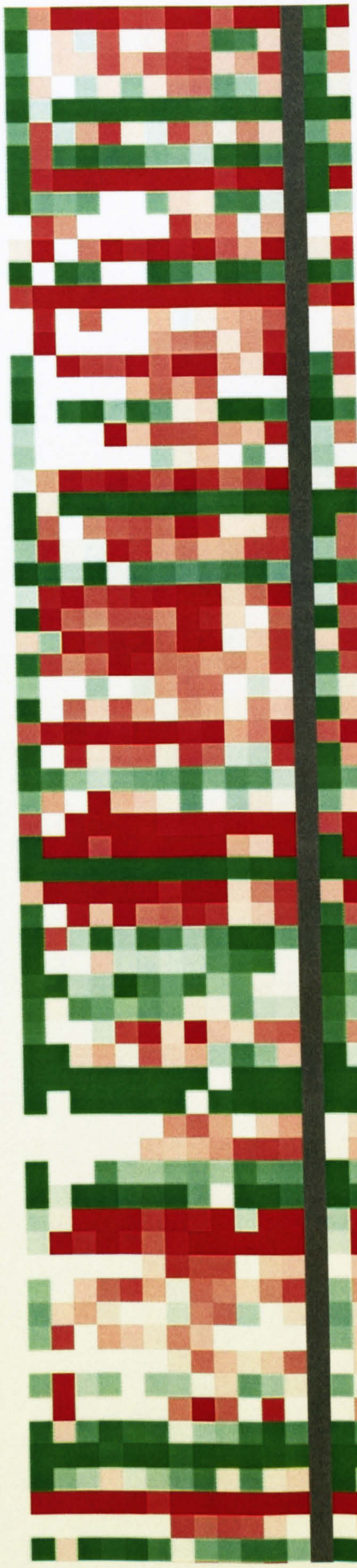

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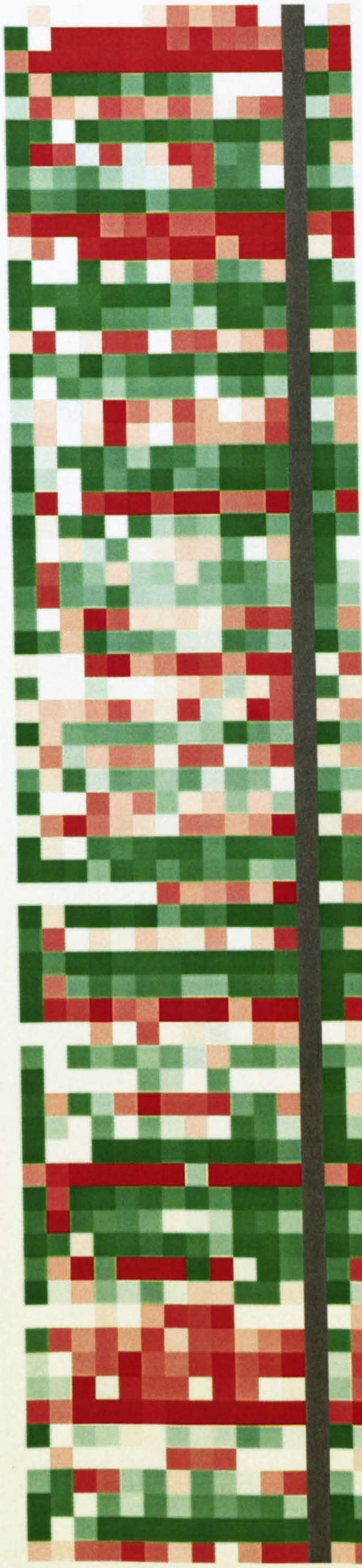

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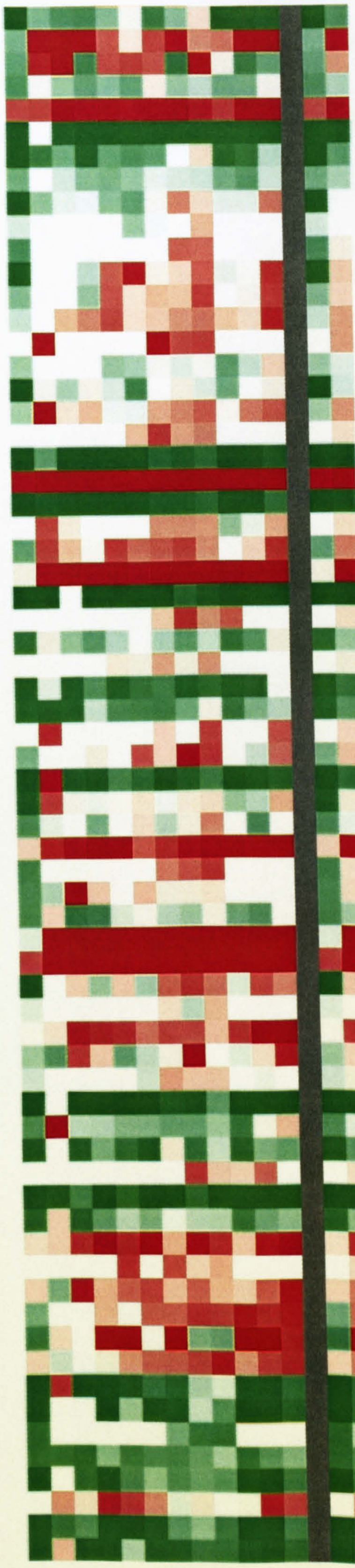
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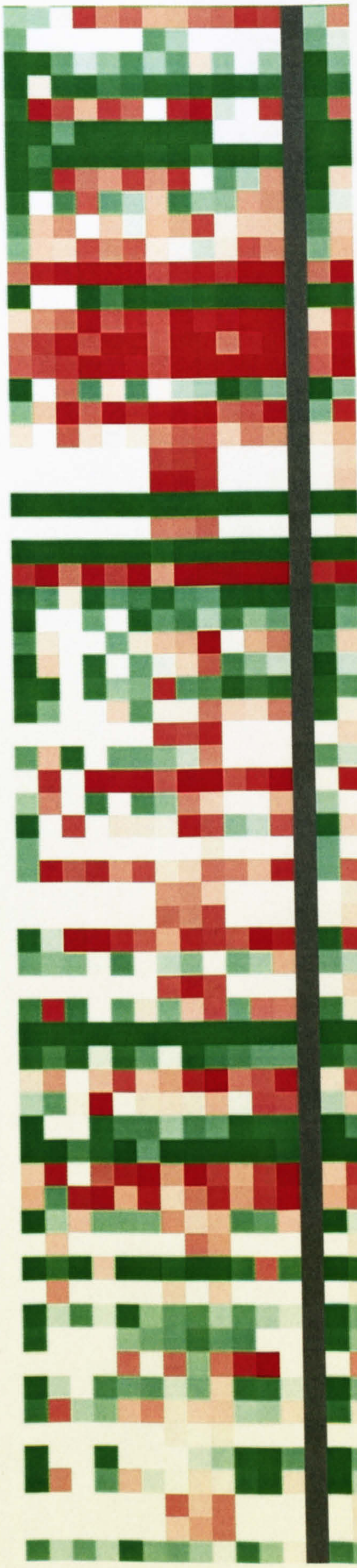
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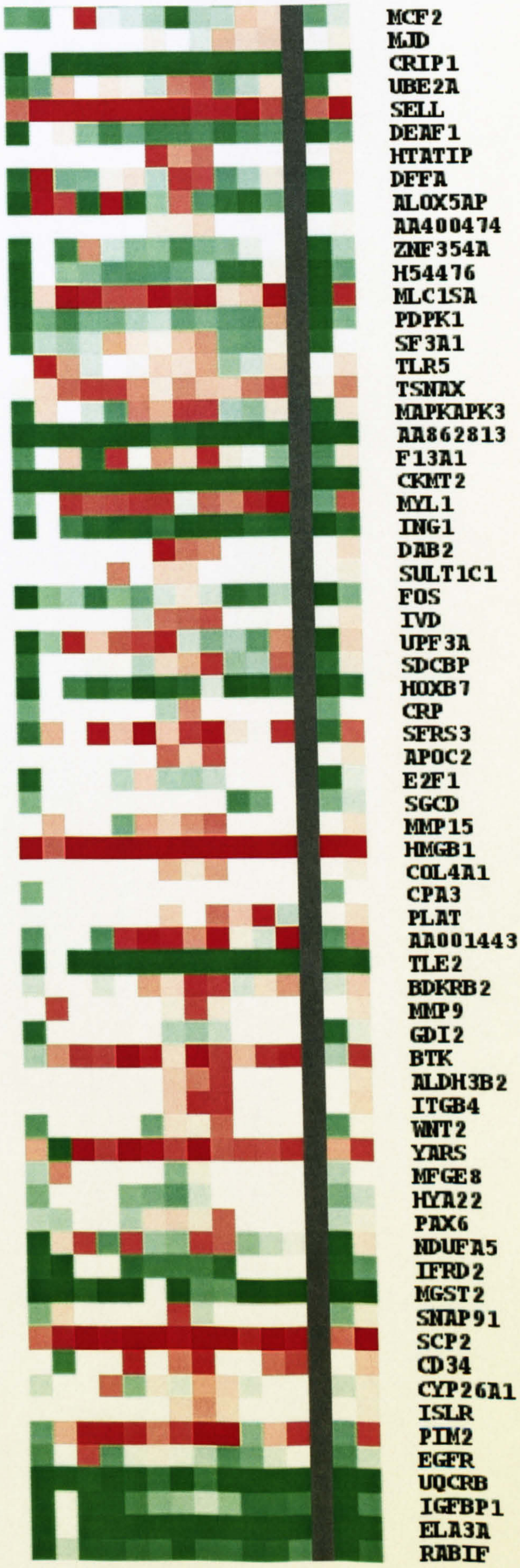
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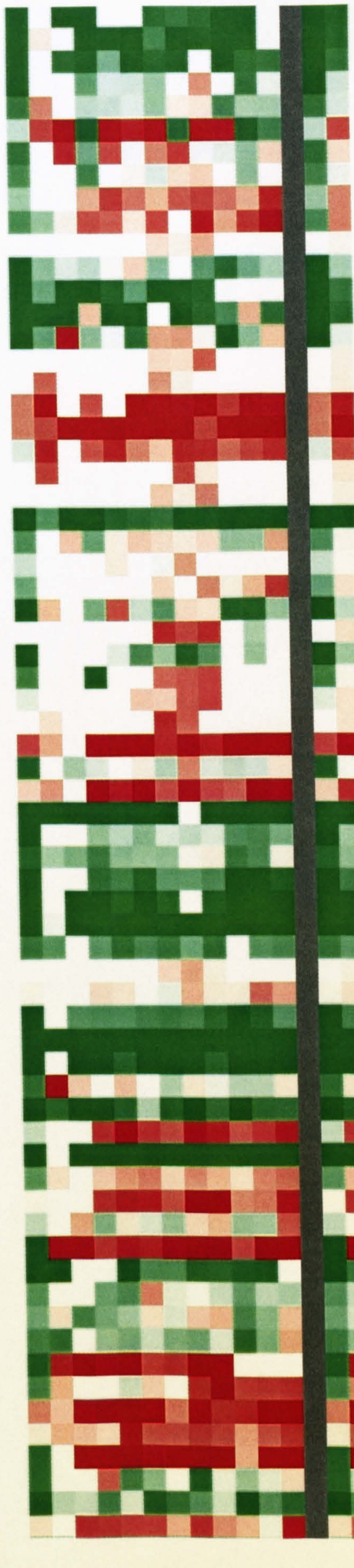
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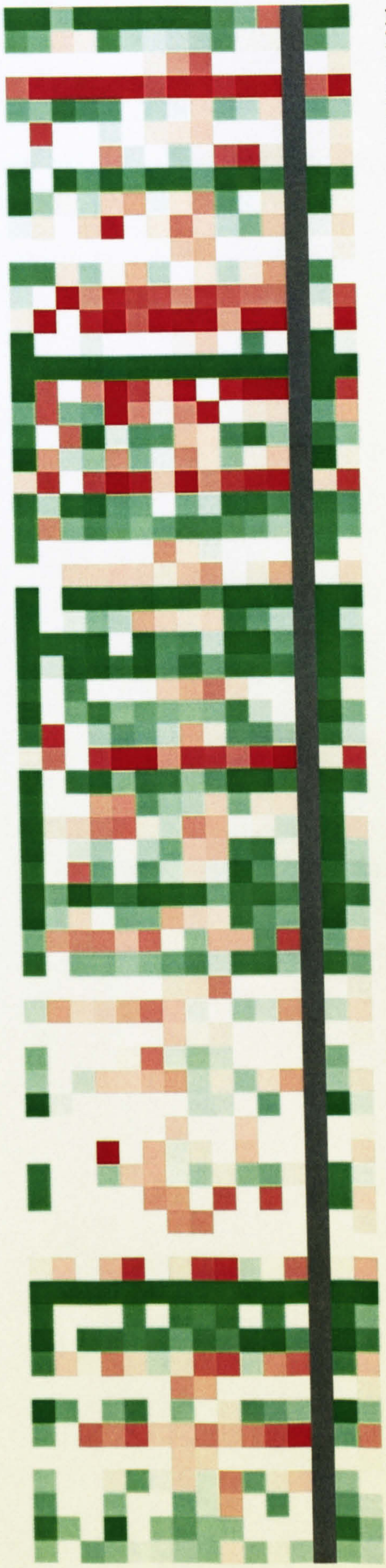
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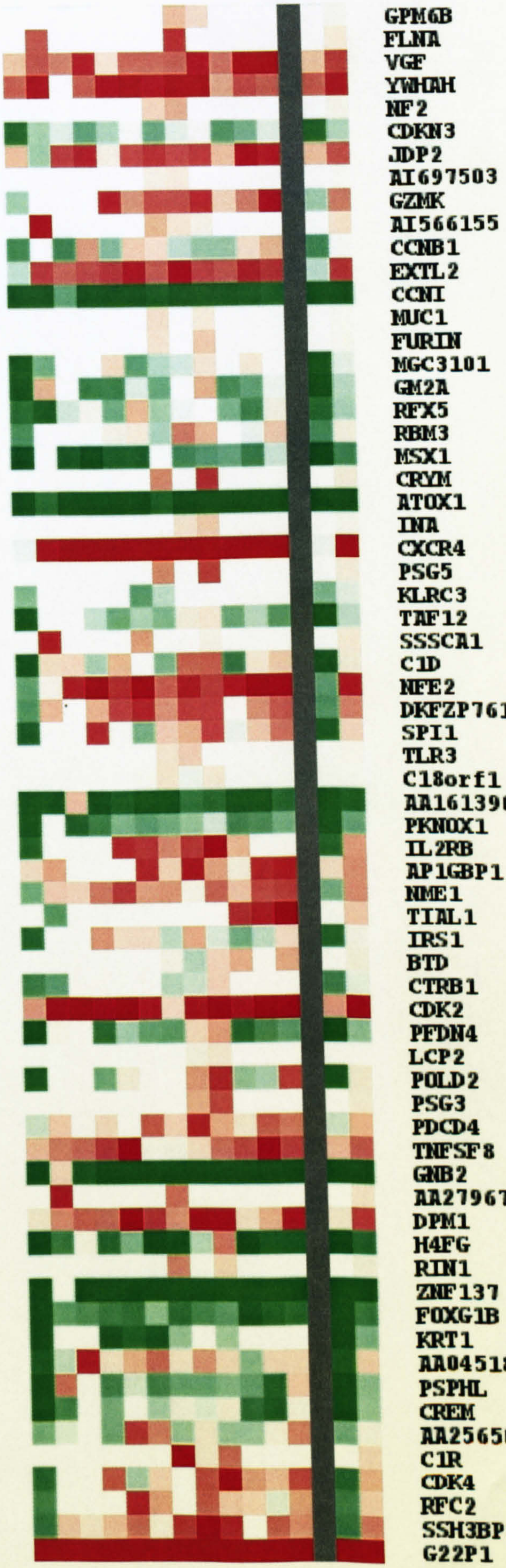
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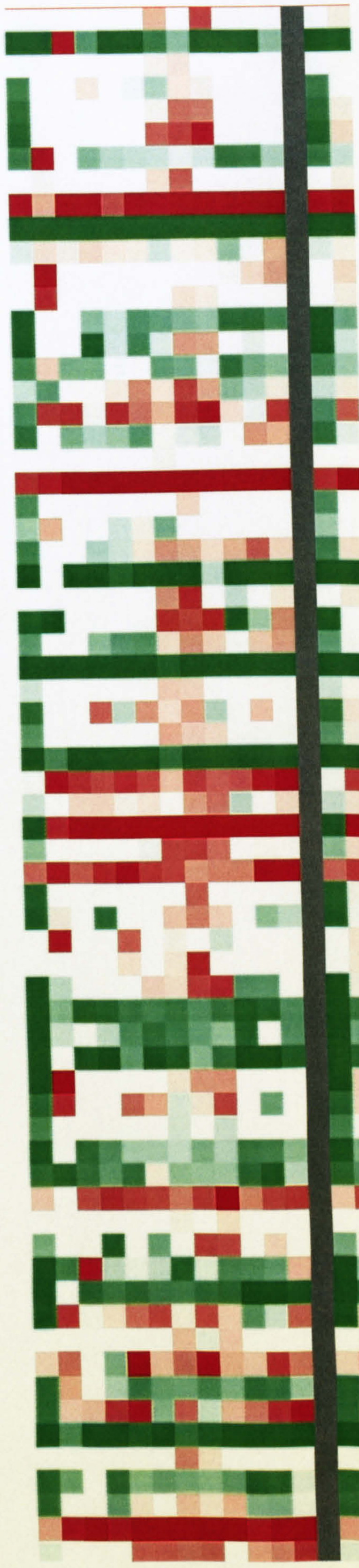

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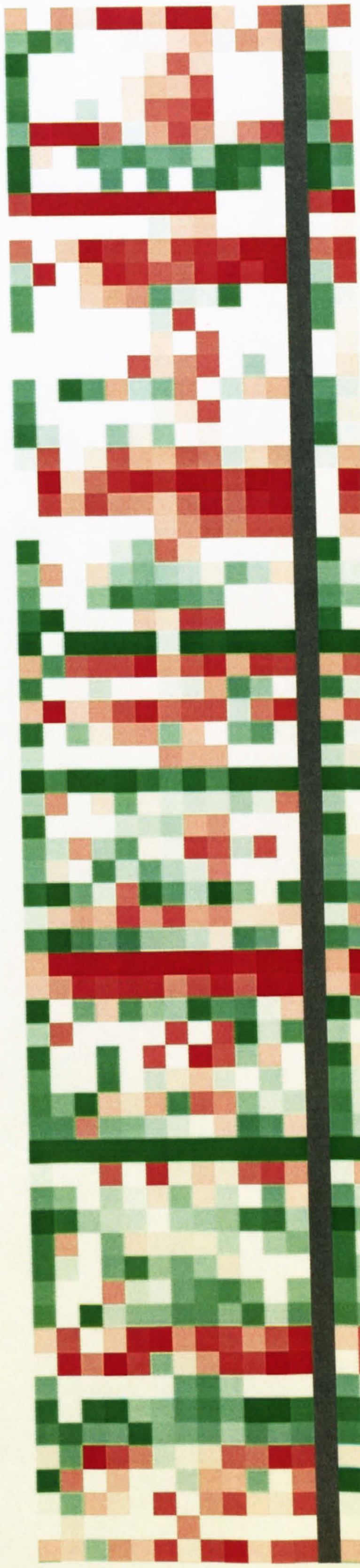

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CGR19
GNG10

Appendix 2

2.1 Table of genes with significantly decreased polysome association in B-CLL cells when compared to GM1953 cells.

Cluster Number	Gene Name	Gene Description
Hs.159557	KPNA2	karyopherin alpha 2 (RAG cohort 1, importin alpha 1)
Hs.173125	PPIF	peptidylprolyl isomerase F (cyclophilin F)
Hs.89643	TKT	transketolase (Wernicke-Korsakoff syndrome)
Hs.2134	TRAF1	TNF receptor-associated factor 1
Hs.182265	KRT19	keratin 19
Hs.278468	PMS2L4	postmeiotic segregation increased 2-like 4
Hs.79005	PTPRK	protein tyrosine phosphatase, receptor type, K
Hs.196384	PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
Hs.83775	DGCR11	DiGeorge syndrome critical region gene 11
Hs.1602	DPYD	dihydropyrimidine dehydrogenase
Hs.4158	REG1B	regenerating islet-derived 1 beta (pancreatic stone protein, pancreatic thread protein)
Hs.77917	UCHL3	ubiquitin carboxyl-terminal esterase L3 (ubiquitin thiolesterase)
Hs.7768	FIBP	fibroblast growth factor (acidic) intracellular binding protein
Hs.433434	PSMB7	proteasome (prosome, macropain) subunit, beta type, 7
Hs.290	PLA2G5	phospholipase A2, group V
Hs.75586	CCND2	cyclin D2
Hs.170808	GAD2	glutamate decarboxylase 2 (pancreatic islets and brain, 65kDa)
Hs.84775	SDNSF	neural stem cell derived neuronal survival protein
Hs.422953	N63107	EST
Hs.149155	VDAC1	voltage-dependent anion channel 1
Hs.82201	CSNK2A2	casein kinase 2, alpha prime polypeptide
Hs.83173	CCND3	cyclin D3
Hs.3631	IGBP1	immunoglobulin (CD79A) binding protein 1
Hs.288856	PFDN5	prefoldin 5
Hs.155433	ATP5C1	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1
Hs.82240	STX3A	syntaxin 3A
Hs.73737	SFRS1	splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)
Hs.79019	BIRC1	baculoviral IAP repeat-containing 1
Hs.149098	SMTN	smoothelin
Hs.13351	LANCL1	LanC lantibiotic synthetase component C-like 1 (bacterial)
Hs.75841	C12orf8	chromosome 12 open reading frame 8
Hs.3164	NUCB2	nucleobindin 2
Hs.75103	YWHAZ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide
Hs.433700	PKIA	protein kinase (cAMP-dependent, catalytic) inhibitor alpha
Hs.63525	PCBP2	poly(rC) binding protein 2
Hs.30054	F5	coagulation factor V (proaccelerin, labile factor)
Hs.86386	MCL1	myeloid cell leukemia sequence 1 (BCL2-related)
Hs.75412	ARMET	arginine-rich, mutated in early stage tumors
Hs.40866	KCNQ3	potassium voltage-gated channel, KQT-like subfamily, member 3
Hs.42957	METTL1	methyltransferase-like 1
Hs.75607	MARCKS	myristoylated alanine-rich protein kinase C substrate
Hs.41707	HSPB3	heat shock 27kDa protein 3
Hs.367676	DUT	dUTP pyrophosphatase

Hs.82043	D123	D123 gene product
Hs.95990	PKLR	pyruvate kinase, liver and RBC
Hs.167584	SLC2A2	solute carrier family 2 (facilitated glucose transporter), member 2
Hs.155202	TCEB3	transcription elongation factor B (SIII), polypeptide 3 (110kDa, elongin A)
Hs.397980	SLC25A6	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6
Hs.394389	PPIB	peptidylprolyl isomerase B (cyclophilin B)
Hs.75184	CHI3L1	chitinase 3-like 1 (cartilage glycoprotein-39)
Hs.3100	KARS	lysyl-tRNA synthetase
Hs.110837	TUBB5	tubulin, beta, 5
Hs.166733	LNPEP	leucyl/cystinyl aminopeptidase
Hs.433326	IGFBP2	insulin-like growth factor binding protein 2, 36kDa
Hs.351316	TM4SF1	transmembrane 4 superfamily member 1
Hs.7984	PSCD3	pleckstrin homology, Sec7 and coiled/coil domains 3
Hs.1770	LIG1	ligase I, DNA, ATP-dependent
Hs.423422	N62761	ESTs, Highly similar to S55330 fragile X mental retardation syndrome related protein FXR1 - human [H.sapiens]
Hs.799	DTR	diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor)
Hs.93213	BAK1	BCL2-antagonist/killer 1
Hs.576	FUCA1	fucosidase, alpha-L- 1, tissue
Hs.167246	POR	P450 (cytochrome) oxidoreductase
Hs.269222	MAPK4	mitogen-activated protein kinase 4
Hs.115263	EREG	epiregulin
Hs.183556	SLC1A5	solute carrier family 1 (neutral amino acid transporter), member 5
Hs.275182	PIP5K1C	phosphatidylinositol-4-phosphate 5-kinase, type I, gamma
Hs.432329	DKFZp564A176	hypothetical protein DKFZp564A176
Hs.348387	GSTM4	glutathione S-transferase M4
Hs.160786	ASS	argininosuccinate synthetase
Hs.82535	SLC6A12	solute carrier family 6 (neurotransmitter transporter, betaine/GABA), member 12
Hs.75812	PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial)
Hs.14155	B7H2	B7-like protein
Hs.1019	PTHR1	parathyroid hormone receptor 1
Hs.313	SPP1	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)
Hs.274348	BAT3	HLA-B associated transcript 3
Hs.166017	MITF	microphthalmia-associated transcription factor
Hs.76392	ALDH1A1	aldehyde dehydrogenase 1 family, member A1
Hs.77356	TFRC	transferrin receptor (p90, CD71)
Hs.118787	TGFBI	transforming growth factor, beta-induced, 68kDa
Hs.73090	NFKB2	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)
Hs.433307	BCKDHA	branched chain keto acid dehydrogenase E1, alpha polypeptide (maple syrup urine disease)
Hs.3712	UQCRFS1	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1
Hs.391270	CRYAB	crystallin, alpha B
Hs.64	SDHB	succinate dehydrogenase complex, subunit B, iron sulfur (Ip)
Hs.89717	CPA2	carboxypeptidase A2 (pancreatic)
Hs.75206	PPP3CC	protein phosphatase 3 (formerly 2B), catalytic subunit, gamma isoform (calcineurin A gamma)
Hs.75574	MRPL19	mitochondrial ribosomal protein L19
Hs.622	BRAF	v-raf murine sarcoma viral oncogene homolog B1
Hs.88474	PTGS1	prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)
Hs.173162	NOC4	neighbor of COX4

Hs.296281	ILF1	interleukin enhancer binding factor 1
Hs.268530	GPS1	G protein pathway suppressor 1
Hs.75231	SLC16A1	solute carrier family 16 (monocarboxylic acid transporters), member 1
Hs.172847	DNAJC4	DnaJ (Hsp40) homolog, subfamily C, member 4
Hs.77542	BG230619	ESTs, Moderately similar to cytokine receptor-like factor 2; cytokine receptor CRL2 precusor [Homo sapiens] [H.sapiens]
Hs.15617	AA017199	ESTs, Weakly similar to hypothetical protein FLJ20378 [Homo sapiens] [H.sapiens]
Hs.68583	MIPEP	mitochondrial intermediate peptidase
Hs.1027	RRAD	Ras-related associated with diabetes
Hs.100669	RAD51L1	RAD51-like 1 (S. cerevisiae)
Hs.146688	PTGES	prostaglandin E synthase
Hs.74335	HSPCB	heat shock 90kDa protein 1, beta
Hs.429366	TXNL	thioredoxin-like, 32kDa
Hs.288991	TNIP2	TNFAIP3 interacting protein 2
Hs.129548	HNRPK	heterogeneous nuclear ribonucleoprotein K
Hs.75297	FGF1	fibroblast growth factor 1 (acidic)
Hs.54089	BARD1	BRCA1 associated RING domain 1
Hs.241572	GOLGA5	golgi autoantigen, golgin subfamily a, 5
Hs.89691	UGT2B4	UDP glycosyltransferase 2 family, polypeptide B4
Hs.80426	BRE	brain and reproductive organ-expressed (TNFRSF1A modulator)
Hs.7953	LOC51125	HSPC041 protein
Hs.1334	MYB	v-myb myeloblastosis viral oncogene homolog (avian)
Hs.19192	CDK2	cyclin-dependent kinase 2
Hs.433845	KRT5	keratin 5 (epidermolysis bullosa simplex, Dowling-Meara/Kobner/Weber-Cockayne types)
Hs.89695	INSR	insulin receptor
Hs.297681	SERPINA1	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
Hs.76038	IDI1	isopentenyl-diphosphate delta isomerase
Hs.507	CDSN	corneodesmosin
Hs.344037	PRC1	protein regulator of cytokinesis 1
Hs.155637	PRKDC	protein kinase, DNA-activated, catalytic polypeptide
Hs.151051	MAPK10	mitogen-activated protein kinase 10
Hs.26	FECH	ferrochelatase (protoporphyria)
Hs.169610	CD44	CD44 antigen (homing function and Indian blood group system)
Hs.172847	DNAJC4	DnaJ (Hsp40) homolog, subfamily C, member 4
Hs.80247	CCK	cholecystokinin
Hs.283677	GTPBP1	GTP binding protein 1
Hs.167246	POR	P450 (cytochrome) oxidoreductase
Hs.410626	AI363781	EST
Hs.79024	HNRPM	heterogeneous nuclear ribonucleoprotein M
Hs.81029	BLVRA	biliverdin reductase A
Hs.78934	MSH2	mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)
Hs.43388	IFRG28	28kD interferon responsive protein
Hs.109225	VCAM1	vascular cell adhesion molecule 1
Hs.76828	GPC5	glypican 5
Hs.22868	PTPN11	protein tyrosine phosphatase, non-receptor type 11 (Noonan syndrome 1)
Hs.399966	H29256	Homo sapiens full length insert cDNA clone YY51E04, mRNA sequence
Hs.433506	AA188155	Homo sapiens, clone IMAGE:3457786, mRNA, mRNA sequence
Hs.424055	T50699	EST, Moderately similar to estrogen receptor binding site associated antigen 9; estrogen receptor-binding fragment-associated gene 9; receptor-binding cancer antigen expressed on SISO cells; cancer associated surface antigen RCAS1 [Homo sapiens] [H.sapien
Hs.75227	NDUFA9	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 9, 39kDa
Hs.1904	PRKCI	protein kinase C, iota

Hs.194638	POLR2D	polymerase (RNA) II (DNA directed) polypeptide D
Hs.182825	RPL35	ribosomal protein L35
Hs.80343	MMP15	matrix metalloproteinase 15 (membrane-inserted)
Hs.432931	MAN1A1	mannosidase, alpha, class 1A, member 1
Hs.72910	CRYGC	crystallin, gamma C
Hs.152978	PSME3	proteasome (prosome, macropain) activator subunit 3 (PA28 gamma; Ki)
Hs.28853	CDC7L1	CDC7 cell division cycle 7-like 1 (S. cerevisiae)
Hs.118825	MAP2K6	mitogen-activated protein kinase kinase 6
Hs.301613	JTV1	JTV1 gene
Hs.1765	LCK	lymphocyte-specific protein tyrosine kinase
Hs.2134	TRAF1	TNF receptor-associated factor 1
Hs.430561	AA598487	EST, Moderately similar to PUR2_HUMAN Trifunctional purine biosynthetic protein adenosine-3 [Includes: Phosphoribosylamine--glycine ligase (GARS) (Glycinamide ribonucleotide synthetase) (Phosphoribosylglycinamide synthetase); Phosphoribosylformylglycinami
Hs.15110	ZNF211	zinc finger protein 211
Hs.90073	CSE1L	CSE1 chromosome segregation 1-like (yeast)
Hs.78672	LAMA4	laminin, alpha 4
Hs.118630	MXI1	MAX interacting protein 1
Hs.183650	CRABP2	cellular retinoic acid binding protein 2
Hs.101448	MTA1	metastasis associated 1
Hs.285115	IL13RA1	interleukin 13 receptor, alpha 1
Hs.20137	BIKE	BMP-2 inducible kinase
Hs.164915	SNAPC3	small nuclear RNA activating complex, polypeptide 3, 50kDa
Hs.406367	AA491213	Unknown (protein for IMAGE:4822098) [Homo sapiens], mRNA sequence
Hs.3132	STAR	steroidogenic acute regulatory protein
Hs.11465	GSTTLp28	glutathione-S-transferase like; glutathione transferase omega
Hs.180062	PSMB8	proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional protease 7)
Hs.279652	MRPL4	mitochondrial ribosomal protein L4
Hs.890	LTB	lymphotoxin beta (TNF superfamily, member 3)
Hs.77060	PSMB6	proteasome (prosome, macropain) subunit, beta type, 6
Hs.74578	DDX9	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 9 (RNA helicase A, nuclear DNA helicase II; leukophysin)
Hs.172458	IDS	iduronate 2-sulfatase (Hunter syndrome)
Hs.19699	CGTHBA	Conserved gene telomeric to alpha globin cluster
Hs.23205	MPP2	membrane protein, palmitoylated 2 (MAGUK p55 subfamily member 2)
Hs.1432	PRKCSH	protein kinase C substrate 80K-H
Hs.172851	ARG2	arginase, type II
Hs.77326	IGFBP3	insulin-like growth factor binding protein 3
Hs.172647	GOLGA1	golgi autoantigen, golgin subfamily a, 1
Hs.388392	R45428	ESTs
Hs.83583	ARPC2	actin related protein 2/3 complex, subunit 2, 34kDa
Hs.101382	TNFAIP2	tumor necrosis factor, alpha-induced protein 2
Hs.423615	CDC34	cell division cycle 34
Hs.288986	SMN1	survival of motor neuron 1, telomeric
Hs.193974	GSR	glutathione reductase
Hs.99987	ERCC2	excision repair cross-complementing rodent repair deficiency, complementation group 2 (xeroderma pigmentosum D)
Hs.74619	PSMD2	proteasome (prosome, macropain) 26S subunit, non-ATPase, 2
Hs.3352	HDAC2	histone deacetylase 2
Hs.7688	PPP2R2B	protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), beta isoform
Hs.78846	HSPB2	heat shock 27kDa protein 2
Hs.305890	BCL2L1	BCL2-like 1

Hs.77498	PRPSAP1	phosphoribosyl pyrophosphate synthetase-associated protein 1
Hs.46	PTAFR	platelet-activating factor receptor
Hs.82251	MYO1E	myosin IE
Hs.1145	WT1	Wilms tumor 1
Hs.278544	ACAT2	acetyl-Coenzyme A acetyltransferase 2 (acetoacetyl Coenzyme A thiolase)
Hs.301746	RAP2A	RAP2A, member of RAS oncogene family
Hs.76688	CES1	carboxylesterase 1 (monocyte/macrophage serine esterase 1)
Hs.173902	PPP2R1A	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), alpha isoform
Hs.1256	ARSB	arylsulfatase B
Hs.155462	MCM6	MCM6 minichromosome maintenance deficient 6 (MIS5 homolog, S. pombe) (S. cerevisiae)
Hs.1063	SNRPC	small nuclear ribonucleoprotein polypeptide C
Hs.376750	AI289598	Homo sapiens cDNA FLJ33408 fis, clone BRACE2010550, highly similar to Human cell growth regulator CGR19 mRNA, mRNA sequence
Hs.927	MYBPH	myosin binding protein H
Hs.8248	NDUFS1	NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa (NADH-coenzyme Q reductase)
Hs.173724	CKB	creatine kinase, brain
Hs.99910	PFKP	phosphofructokinase, platelet
Hs.21639	APEG1	nuclear protein, marker for differentiated aortic smooth muscle and down-regulated with vascular injury
Hs.352231	AA464256	PP3731 [Homo sapiens], mRNA sequence
Hs.278672	M11S1	membrane component, chromosome 11, surface marker 1
Hs.25156	PPI5PIV	phosphatidylinositol (4,5) bisphosphate 5-phosphatase homolog; phosphatidylinositol polyphosphate 5-phosphatase type IV
Hs.79335	SMARCD1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 1
Hs.166091	LIG4	ligase IV, DNA, ATP-dependent
Hs.64794	ZNF183	zinc finger protein 183 (RING finger, C3HC4 type)
Hs.50964	CEACAM1	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
Hs.12553	R39258	ESTs
Hs.351863	TST	thiosulfate sulfurtransferase (rhodanese)
Hs.324787	AA490044	Homo sapiens G21VN02 mRNA, mRNA sequence
Hs.171734	PPP2R5C	protein phosphatase 2, regulatory subunit B (B56), gamma isoform
Hs.183153	ARF4L	ADP-ribosylation factor 4-like
Hs.2055	UBE1	ubiquitin-activating enzyme E1 (A1S9T and BN75 temperature sensitivity complementing)
Hs.261927	PSMB5	proteasome (prosome, macropain) subunit, beta type, 5
Hs.809	HGF	hepatocyte growth factor (hepapoietin A; scatter factor)
Hs.81221	AA505045	Human CLL-27 transcript of unrearranged immunoglobulin V(H)5 pseudogene, mRNA sequence
Hs.199248	PTGER4	prostaglandin E receptor 4 (subtype EP4)
Hs.112058	SIVA	CD27-binding (Siva) protein
Hs.155024	BCL6	B-cell CLL/lymphoma 6 (zinc finger protein 51)
Hs.1259	ASGR2	asialoglycoprotein receptor 2
Hs.246857	MAPK9	mitogen-activated protein kinase 9
Hs.349110	MST1	macrophage stimulating 1 (hepatocyte growth factor-like)
Hs.158315	IL18RAP	interleukin 18 receptor accessory protein
Hs.74076	CD163	CD163 antigen
Hs.166071	CDK5	cyclin-dependent kinase 5
Hs.77637	HOXA4	homeo box A4
Hs.155119	EHD1	EH-domain containing 1
Hs.106876	ATP6V0D1	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d isoform 1
Hs.100764	CTSG	cathepsin G

Hs.35937	MYF6	myogenic factor 6 (herculin)
Hs.256278	TNFRSF1B	tumor necrosis factor receptor superfamily, member 1B
Hs.7779	FLJ12619	hypothetical protein FLJ12619
Hs.421650	H99364	EST
Hs.169992	SDBCAG84	serologically defined breast cancer antigen 84
Hs.423615	CDC34	cell division cycle 34
Hs.78944	RGS2	regulator of G-protein signalling 2, 24kDa
Hs.44708	PK428	Ser-Thr protein kinase related to the myotonic dystrophy protein kinase
Hs.71992	APG-1	heat shock protein (hsp110 family)
Hs.77840	ANXA4	annexin A4
Hs.656	CDC25C	cell division cycle 25C
Hs.22826	TMOD3	tropomodulin 3 (ubiquitous)
Hs.183671	TDO2	tryptophan 2,3-dioxygenase
Hs.195432	ALDH2	aldehyde dehydrogenase 2 family (mitochondrial)
Hs.89663	CYP24	cytochrome P450, subfamily XXIV (vitamin D 24-hydroxylase)
Hs.99936	KRT10	keratin 10 (epidermolytic hyperkeratosis; keratosis palmaris et plantaris)
Hs.239818	PIK3CB	phosphoinositide-3-kinase, catalytic, beta polypeptide
Hs.2227	CEBPG	CCAAT/enhancer binding protein (C/EBP), gamma
Hs.227913	API5	apoptosis inhibitor 5
Hs.79474	YWHAE	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide
Hs.81942	POLA2	polymerase (DNA-directed), alpha (70kD)
Hs.389277	ARFRP1	ADP-ribosylation factor related protein 1
Hs.79391	HD	huntingtin (Huntington disease)
Hs.25648	TNFRSF5	tumor necrosis factor receptor superfamily, member 5
Hs.386741	ANXA7	annexin A7
Hs.78580	DDX1	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 1
Hs.89866	CPO	coproporphyrinogen oxidase (coproporphyria, harderoporphyria)
Hs.380447	ATP1A3	ATPase, Na+/K+ transporting, alpha 3 polypeptide
Hs.4756	FEN1	flap structure-specific endonuclease 1
Hs.382777	AA644191	ESTs, Moderately similar to A54869 ADP-ribosylation factor-like 3 - human [H.sapiens]
Hs.75655	P4HB	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), beta polypeptide (protein disulfide isomerase; thyroid hormone binding protein p55)
Hs.94672	GCN5L1	GCN5 general control of amino-acid synthesis 5-like 1 (yeast)
Hs.118640	DVL2	dishevelled, dsh homolog 2 (Drosophila)
Hs.165843	CSNK2B	casein kinase 2, beta polypeptide
Hs.288856	PFDN5	prefoldin 5
Hs.21595	DXYS155E	DNA segment on chromosome X and Y (unique) 155 expressed sequence
Hs.21254	TRIP	TRAF interacting protein
Hs.343874	PRODH	proline dehydrogenase (oxidase) 1
Hs.74626	AP2B1	adaptor-related protein complex 2, beta 1 subunit
Hs.406111	MYO1C	myosin IC
Hs.392837	PGD	phosphogluconate dehydrogenase
Hs.279929	HSGP25L2G	gp25L2 protein
Hs.1432	PRKCSH	protein kinase C substrate 80K-H
Hs.70500	RANBP20	RAN binding protein 20
Hs.85137	CCNA2	cyclin A2
Hs.79358	TESK1	testis-specific kinase 1
Hs.240443	AA400234	Homo sapiens cDNA: FLJ23538 fis, clone LNG08010, highly similar to BETA2 Human MEN1 region clone epsilon/beta mRNA, mRNA sequence
Hs.78864	FCGR2A	Fc fragment of IgG, low affinity IIa, receptor for (CD32)

Hs.101299	CUL5	cullin 5
Hs.227823	PM5	pM5 protein
Hs.288181	CTSH	cathepsin H
Hs.37055	FGF5	fibroblast growth factor 5
Hs.418489	AA460728	Homo sapiens cDNA FLJ38479 fis, clone FEBRA2022787, mRNA sequence
Hs.79339	LGALS3BP	lectin, galactoside-binding, soluble, 3 binding protein
Hs.3416	ADFP	adipose differentiation-related protein
Hs.9700	CCNE1	cyclin E1
Hs.220529	CEACAM5	carcinoembryonic antigen-related cell adhesion molecule 5
Hs.173594	SERPINF1	serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1
Hs.75586	CCND2	cyclin D2
Hs.4742	GPAA1	GPAA1P anchor attachment protein 1 homolog (yeast)
Hs.17518	cig5	vipirin
Hs.1349	CSF2	colony stimulating factor 2 (granulocyte-macrophage)
Hs.5662	GNB2L1	guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1
Hs.2399	MMP14	matrix metalloproteinase 14 (membrane-inserted)
Hs.172550	PTBP1	polypyrimidine tract binding protein 1
Hs.339	P2RY2	purinergic receptor P2Y, G-protein coupled, 2
Hs.9661	PSMB10	proteasome (prosome, macropain) subunit, beta type, 10
Hs.77448	ALDH4A1	aldehyde dehydrogenase 4 family, member A1
Hs.75339	INPPL1	inositol polyphosphate phosphatase-like 1
Hs.16269	BCL7B	B-cell CLL/lymphoma 7B
Hs.127826	EPOR	erythropoietin receptor
Hs.173497	SEC23B	Sec23 homolog B (S. cerevisiae)
Hs.78406	PIP5K1B	phosphatidylinositol-4-phosphate 5-kinase, type I, beta
Hs.169241	ELK4	ELK4, ETS-domain protein (SRF accessory protein 1)
Hs.79172	SLC25A5	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5
Hs.434043	AA430574	ESTs, Highly similar to A55933 paxillin - human [H.sapiens]
Hs.279474	MKRN2	makorin, ring finger protein, 2
Hs.77628	STARD3	START domain containing 3
Hs.169681	DEDD	death effector domain containing
Hs.174071	GYG	glycogenin
Hs.406186	SF3B4	splicing factor 3b, subunit 4, 49kDa
Hs.347349	CPR2	cell cycle progression 2 protein
Hs.82587	PLD1	phospholipase D1, phophatidylcholine-specific
Hs.80917	AP3S1	adaptor-related protein complex 3, sigma 1 subunit
Hs.72927	IL7	interleukin 7
Hs.82327	GSS	glutathione synthetase
Hs.349650	RAF1	v-raf-1 murine leukemia viral oncogene homolog 1
Hs.184270	CAPZA1	capping protein (actin filament) muscle Z-line, alpha 1
Hs.2012	TCN1	transcobalamin I (vitamin B12 binding protein, R binder family)
Hs.380901	GPS2	G protein pathway suppressor 2
Hs.433619	PLK	polo-like kinase (Drosophila)
Hs.301961	GSTM1	glutathione S-transferase M1
Hs.433506	AA188155	Homo sapiens, clone IMAGE:3457786, mRNA, mRNA sequence
Hs.427785	T52830	Homo sapiens full length insert cDNA clone YA81B05, mRNA sequence
Hs.184402	CAMK1	calcium/calmodulin-dependent protein kinase I
Hs.3764	GUK1	guanylate kinase 1
Hs.75860	HADHA	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit
Hs.367900	PDCD2	programmed cell death 2
Hs.154138	CHI3L2	chitinase 3-like 2

Hs.159971	SMARCB1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1
Hs.21635	TUBG1	tubulin, gamma 1
Hs.234569	ZAP70	zeta-chain (TCR) associated protein kinase 70kDa
Hs.86386	MCL1	myeloid cell leukemia sequence 1 (BCL2-related)
Hs.78867	PTPRZ1	protein tyrosine phosphatase, receptor-type, Z polypeptide 1
Hs.12152	APMCF1	APMCF1 protein
Hs.107474	NAB1	NGFI-A binding protein 1 (EGR1 binding protein 1)
Hs.432833	UBE2I	ubiquitin-conjugating enzyme E2I (UBC9 homolog, yeast)
Hs.334895	RPL10A	ribosomal protein L10a
Hs.77329	PTDSS1	phosphatidylserine synthase 1
Hs.172673	AHCY	S-adenosylhomocysteine hydrolase
Hs.2664	FMO4	flavin containing monooxygenase 4
Hs.356176	ACAA2	acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)
Hs.86958	IFNAR2	interferon (alpha, beta and omega) receptor 2
Hs.77367	CXCL9	chemokine (C-X-C motif) ligand 9
Hs.203238	PDE1B	phosphodiesterase 1B, calmodulin-dependent
Hs.85201	CLECSF2	C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 2 (activation-induced)
Hs.121001	AA009609	Homo sapiens cDNA FLJ20444 fis, clone KAT05128, mRNA sequence
Hs.149923	XBP1	X-box binding protein 1
Hs.18508	GLYAT	glycine-N-acyltransferase
Hs.63525	PCBP2	poly(rC) binding protein 2
Hs.270833	AREG	amphiregulin (schwannoma-derived growth factor)
Hs.3727	UNRIP	unr-interacting protein
Hs.247551	MTX1	metaxin 1
Hs.146861	FLJ20580	hypothetical protein FLJ20580
Hs.155392	CRMP1	collapsin response mediator protein 1
Hs.40500	RER1	similar to S. cerevisiae RER1
Hs.5753	IMPA2	inositol(myo)-1(or 4)-monophosphatase 2
Hs.79357	PSMC6	proteasome (prosome, macropain) 26S subunit, ATPase, 6
Hs.3057	ZNF74	zinc finger protein 74 (Cos52)
Hs.25913	PEX12	peroxisomal biogenesis factor 12
Hs.19710	SLC17A2	solute carrier family 17 (sodium phosphate), member 2
Hs.102135	SSR4	signal sequence receptor, delta (translocon-associated protein delta)
Hs.375108	CD24	CD24 antigen (small cell lung carcinoma cluster 4 antigen)
Hs.323477	SLC16A3	solute carrier family 16 (monocarboxylic acid transporters), member 3
Hs.76084	LMNB2	lamin B2
Hs.82979	MAP4K2	mitogen-activated protein kinase kinase kinase kinase 2
Hs.75160	PFKM	phosphofructokinase, muscle
Hs.62661	GBP1	guanylate binding protein 1, interferon-inducible, 67kDa
Hs.75199	PPP2R5B	protein phosphatase 2, regulatory subunit B (B56), beta isoform
Hs.36451	GRIN2C	glutamate receptor, ionotropic, N-methyl D-aspartate 2C
Hs.110	KIAA0436	putative L-type neutral amino acid transporter
Hs.72988	STAT2	signal transducer and activator of transcription 2, 113kDa
Hs.309763	GRSF1	G-rich RNA sequence binding factor 1
Hs.2704	GPX2	glutathione peroxidase 2 (gastrointestinal)
Hs.416739	IGFBP5	insulin-like growth factor binding protein 5
Hs.82314	HPRT1	hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome)
Hs.400353	DDIT3	DNA-damage-inducible transcript 3
Hs.432330	RAGA	Ras-related GTP-binding protein
Hs.75873	ZYX	zyxin
Hs.75180	PPP5C	protein phosphatase 5, catalytic subunit
Hs.74635	DLD	dihydrolipoamide dehydrogenase (E3 component of pyruvate dehydrogenase complex, 2-oxo-glutarate complex, branched chain keto acid dehydrogenase complex)
Hs.50727	NAGLU	N-acetylglucosaminidase, alpha- (Sanfilippo disease IIIB)

Hs.100729	POU2F1	POU domain, class 2, transcription factor 1
Hs.3833	PAPSS1	3'-phosphoadenosine 5'-phosphosulfate synthase 1
Hs.2853	PCBP1	poly(rC) binding protein 1
Hs.78915	GABPB1	GA binding protein transcription factor, beta subunit 1, 53kDa
Hs.155247	ALDOC	aldolase C, fructose-bisphosphate
Hs.173894	CSF1	colony stimulating factor 1 (macrophage)
Hs.108642	ZNF22	zinc finger protein 22 (KOX 15)
Hs.77490	GSTT1	glutathione S-transferase theta 1
Hs.388862	RPL13	ribosomal protein L13
Hs.134514	ABCA7	ATP-binding cassette, sub-family A (ABC1), member 7
Hs.1360	CYP2B6	cytochrome P450, subfamily IIB (phenobarbital-inducible), polypeptide 6
Hs.82280	RGS10	regulator of G-protein signalling 10
Hs.278581	FGFR2	fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome)
Hs.274382	PRKR	protein kinase, interferon-inducible double stranded RNA dependent
Hs.75335	GATM	glycine amidinotransferase (L-arginine:glycine amidinotransferase)
Hs.27744	RAB3A	RAB3A, member RAS oncogene family
Hs.77100	GTF2E2	general transcription factor IIE, polypeptide 2, beta 34kDa
Hs.122511	CETN1	centrin, EF-hand protein, 1
Hs.154721	ACO1	aconitase 1, soluble
Hs.75290	ARF4	ADP-ribosylation factor 4
Hs.55879	ABCC10	ATP-binding cassette, sub-family C (CFTR/MRP), member 10
Hs.21704	TCF12	transcription factor 12 (HTF4, helix-loop-helix transcription factors 4)
Hs.180655	STK12	serine/threonine kinase 12
Hs.155185	COVA1	cytosolic ovarian carcinoma antigen 1
Hs.75671	STX1A	syntaxin 1A (brain)
Hs.146580	ENO2	enolase 2, (gamma, neuronal)
Hs.5337	IDH2	isocitrate dehydrogenase 2 (NADP+), mitochondrial
Hs.132834	HEM1	hematopoietic protein 1
Hs.194772	OMG	oligodendrocyte myelin glycoprotein
Hs.2879	CPA1	carboxypeptidase A1 (pancreatic)
Hs.75248	TOP2B	topoisomerase (DNA) II beta 180kDa
Hs.14611	DUSP11	dual specificity phosphatase 11 (RNA/RNP complex 1-interacting)
Hs.89582	GRIA2	glutamate receptor, ionotropic, AMPA 2
Hs.162808	PIK3CD	phosphoinositide-3-kinase, catalytic, delta polypeptide
Hs.374466	WARS	tryptophanyl-tRNA synthetase
Hs.267289	POLA	polymerase (DNA directed), alpha
Hs.31218	SCAMP1	secretory carrier membrane protein 1
Hs.82932	CCND1	cyclin D1 (PRAD1: parathyroid adenomatosis 1)
Hs.170197	GOT2	glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2)
Hs.108104	UBE2L3	ubiquitin-conjugating enzyme E2L 3
Hs.74137	TMP21	transmembrane trafficking protein
Hs.51120	CAMP	cathelicidin antimicrobial peptide
Hs.52002	CD5L	CD5 antigen-like (scavenger receptor cysteine rich family)
Hs.82741	PRIM1	primase, polypeptide 1, 49kDa
Hs.380135	FABP1	fatty acid binding protein 1, liver
Hs.151738	MMP9	matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)
Hs.125845	RPE	ribulose-5-phosphate-3-epimerase
Hs.4835	EIF3S8	eukaryotic translation initiation factor 3, subunit 8, 110kDa
Hs.80680	MVP	major vault protein
Hs.748	FGFR1	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)
Hs.89709	GCLM	glutamate-cysteine ligase, modifier subunit
Hs.192023	EIF3S2	eukaryotic translation initiation factor 3, subunit 2 beta, 36kDa

Hs.23960	CCNB1	cyclin B1
Hs.83849	FLII	flightless I homolog (Drosophila)
Hs.22554	HOXB5	homeo box B5
Hs.62187	PIGK	phosphatidylinositol glycan, class K
Hs.117546	NNAT	neuronatin
Hs.288433	HNT	neurotrimin
Hs.40300	CAPN3	calpain 3, (p94)
Hs.351808	FGL2	fibrinogen-like 2
Hs.73853	BMP2	bone morphogenetic protein 2
Hs.396547	H72030	Homo sapiens full length insert cDNA clone YS16F09, mRNA sequence
Hs.169294	TCF7	transcription factor 7 (T-cell specific, HMG-box)
Hs.2157	WAS	Wiskott-Aldrich syndrome (eczema-thrombocytopenia)
Hs.316	DDX6	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 6 (RNA helicase, 54kDa)
Hs.91143	JAG1	jagged 1 (Alagille syndrome)
Hs.373499	NSAP1	NS1-associated protein 1
Hs.77631	GCSH	glycine cleavage system protein H (aminomethyl carrier)
Hs.7510	MAP3K7	mitogen-activated protein kinase kinase kinase 7
Hs.48375	SNRPN	small nuclear ribonucleoprotein polypeptide N
Hs.8375	TRAF4	TNF receptor-associated factor 4
Hs.82542	AOAH	acyloxyacyl hydrolase (neutrophil)
Hs.75593	UROS	uroporphyrinogen III synthase (congenital erythropoietic porphyria)
Hs.75140	LRPAP1	low density lipoprotein-related protein-associated protein 1 (alpha-2-macroglobulin receptor-associated protein 1)
Hs.89555	HCK	hemopoietic cell kinase
Hs.75737	PCM1	pericentriolar material 1
Hs.105097	TK1	thymidine kinase 1, soluble
Hs.122579	ECT2	epithelial cell transforming sequence 2 oncogene
Hs.12013	ABCE1	ATP-binding cassette, sub-family E (OABP), member 1
Hs.181345	SAH	SA hypertension-associated homolog (rat)
Hs.272688	FLJ20397	hypothetical protein FLJ20397
Hs.2910	PRPS2	phosphoribosyl pyrophosphate synthetase 2
Hs.79334	NFIL3	nuclear factor, interleukin 3 regulated
Hs.44087	KIAA1126	KIAA1126 protein
Hs.334718	EFG2	mitochondrial elongation factor G2
Hs.81170	PIM1	pim-1 oncogene
Hs.89657	TAF10	TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 30kDa
Hs.51299	NDUFV2	NADH dehydrogenase (ubiquinone) flavoprotein 2, 24kDa
Hs.226213	CYP51	cytochrome P450, 51 (lanosterol 14-alpha-demethylase)
Hs.310512	CCRL1	chemokine (C-C motif) receptor-like 1
Hs.154207	CENPC1	centromere protein C 1
Hs.169919	ETFA	electron-transfer-flavoprotein, alpha polypeptide (glutaric aciduria II)
Hs.69563	CDC6	CDC6 cell division cycle 6 homolog (S. cerevisiae)
Hs.75257	SLBP	stem-loop (histone) binding protein
Hs.82772	COL11A1	collagen, type XI, alpha 1
Hs.193400	IL6R	interleukin 6 receptor
Hs.301055	C20orf104	chromosome 20 open reading frame 104
Hs.38069	C8B	complement component 8, beta polypeptide
Hs.82906	CDC20	CDC20 cell division cycle 20 homolog (S. cerevisiae)
Hs.433619	PLK	polo-like kinase (Drosophila)
Hs.22919	FUS2	putative tumor suppressor
Hs.313342	CD47	CD47 antigen (Rh-related antigen, integrin-associated signal transducer)
Hs.388	NUDT1	nudix (nucleoside diphosphate linked moiety X)-type motif 1
Hs.89768	GABRB1	gamma-aminobutyric acid (GABA) A receptor, beta 1
Hs.1288	ACTA1	actin, alpha 1, skeletal muscle

Hs.158460	CDK5R2	cyclin-dependent kinase 5, regulatory subunit 2 (p39)
Hs.89525	HDGF	hepatoma-derived growth factor (high-mobility group protein 1-like)
Hs.433813	PIG11	p53-induced protein
Hs.112255	NUP98	nucleoporin 98kDa
Hs.76494	PRELP	proline arginine-rich end leucine-rich repeat protein
Hs.42945	ASM3A	acid sphingomyelinase-like phosphodiesterase
Hs.83753	SNRPB	small nuclear ribonucleoprotein polypeptides B and B1
Hs.87247	HRK	harakiri, BCL2 interacting protein (contains only BH3 domain)
Hs.323079	R14294	Homo sapiens mRNA; cDNA DKFZp564P116 (from clone DKFZp564P116), mRNA sequence
Hs.151641	GARP	glycoprotein A repetitions predominant
Hs.157091	IVL	involucrin
Hs.173554	UQCRC2	ubiquinol-cytochrome c reductase core protein II
Hs.75087	FASTK	FAST kinase
Hs.146381	RBMX	RNA binding motif protein, X chromosome
Hs.33084	SLC2A5	solute carrier family 2 (facilitated glucose/fructose transporter), member 5
Hs.77439	PRKAR2B	protein kinase, cAMP-dependent, regulatory, type II, beta
Hs.73828	TAL1	T-cell acute lymphocytic leukemia 1
Hs.166154	JAG2	jagged 2
Hs.89751	MS4A1	membrane-spanning 4-domains, subfamily A, member 1
Hs.434026	AA812979	Homo sapiens, clone IMAGE:3862468, mRNA, mRNA sequence
Hs.77100	GTF2E2	general transcription factor IIE, polypeptide 2, beta 34kDa
Hs.79162	SSRP1	structure specific recognition protein 1
Hs.112058	SIVA	CD27-binding (Siva) protein
Hs.397221	N26769	EST, Highly similar to A41230 DNA-3-methyladenine glycosidase I (EC 3.2.2.20) - human [H.sapiens]
Hs.277445	DGKZ	diacylglycerol kinase, zeta 104kDa
Hs.250712	CACNB3	calcium channel, voltage-dependent, beta 3 subunit
Hs.1314	TNFRSF8	tumor necrosis factor receptor superfamily, member 8
Hs.202384	T54	T54 protein
Hs.288261	MADH3	MAD, mothers against decapentaplegic homolog 3 (Drosophila)
Hs.185973	DEGS	degenerative spermatocyte homolog, lipid desaturase (Drosophila)
Hs.152720	MPHOSPH 6	M-phase phosphoprotein 6
Hs.196209	RAE1	RAE1 RNA export 1 homolog (S. pombe)
Hs.153752	CDC25B	cell division cycle 25B
Hs.82919	CUL2	cullin 2
Hs.46319	SHBG	sex hormone-binding globulin
Hs.99853	FBL	fibrillarin
Hs.360791	H28344	ESTs, Highly similar to S17361 transcription elongation factor IIS (clone pHIIS 1) - human [H.sapiens]
Hs.90370	ARPC1A	actin related protein 2/3 complex, subunit 1A, 41kDa
Hs.260622	HSPC121	butyrate-induced transcript 1
Hs.155165	ZFPL1	zinc finger protein-like 1
Hs.173824	TDG	thymine-DNA glycosylase
Hs.71891	DDR2	discoidin domain receptor family, member 2
Hs.1519	PRKAR1B	protein kinase, cAMP-dependent, regulatory, type I, beta
Hs.82173	TIEG	TGFB inducible early growth response
Hs.417533	H17528	EST
Hs.2969	SKI	v-ski sarcoma viral oncogene homolog (avian)
Hs.78793	PRKCZ	protein kinase C, zeta
Hs.8047	FANCG	Fanconi anemia, complementation group G
Hs.75074	MAPKAPK 2	mitogen-activated protein kinase-activated protein kinase 2
Hs.169998	BST1	bone marrow stromal cell antigen 1
Hs.79380	PWP2H	PWP2 periodic tryptophan protein homolog (yeast)
Hs.6453	ITPK1	inositol 1,3,4-triphosphate 5/6 kinase

Hs.152096	CYP2J2	cytochrome P450, subfamily IIJ (arachidonic acid epoxygenase) polypeptide 2
Hs.155560	CANX	calnexin
Hs.75431	FGG	fibrinogen, gamma polypeptide
Hs.3697	AGT	angiotensinogen (serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 8)
Hs.1098	ERdj5	ER-resident protein ERdj5
Hs.5398	GMPS	guanine monphosphate synthetase
Hs.204041	C14orf3	chromosome 14 open reading frame 3
Hs.278568	HFL1	H factor (complement)-like 1
Hs.151123	SHC3	neuronal Shc
Hs.211595	PTPN13	protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phosphatase)
Hs.74375	DVL1	dishevelled, dsh homolog 1 (Drosophila)
Hs.300471	SEC13L1	SEC13-like 1 (S. cerevisiae)
Hs.2717	SPAG11	sperm associated antigen 11
Hs.28081	EIF3S4	eukaryotic translation initiation factor 3, subunit 4 delta, 44kDa
Hs.171075	RFC5	replication factor C (activator 1) 5, 36.5kDa
Hs.129953	EWSR1	Ewing sarcoma breakpoint region 1
Hs.29656	CDKN2D	cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4)
Hs.82359	TNFRSF6	tumor necrosis factor receptor superfamily, member 6
Hs.24301	POLR2E	polymerase (RNA) II (DNA directed) polypeptide E, 25kDa
Hs.153487	STAM	signal transducing adaptor molecule (SH3 domain and ITAM motif) 1
Hs.143102	AOC2	amine oxidase, copper containing 2 (retina-specific)
Hs.376447	P38IP	transcription factor (p38 interacting protein)
Hs.110299	MAP2K7	mitogen-activated protein kinase kinase 7
Hs.58169	HEC	highly expressed in cancer, rich in leucine heptad repeats
Hs.6557	T64057	Homo sapiens cDNA FLJ25295 fis, clone STM07531, mRNA sequence
Hs.1691	GBE1	glucan (1,4-alpha-), branching enzyme 1 (glycogen branching enzyme, Andersen disease, glycogen storage disease type IV)
Hs.106185	RALGDS	ral guanine nucleotide dissociation stimulator
Hs.94308	RAB35	RAB35, member RAS oncogene family
Hs.347353	NR1H3	nuclear receptor subfamily 1, group H, member 3
Hs.75189	DAP	death-associated protein
Hs.21537	PPP1CB	protein phosphatase 1, catalytic subunit, beta isoform
Hs.351474	FLJ30002	hypothetical protein FLJ30002
Hs.119187	AA411169	Unknown (protein for IMAGE:4827714) [Homo sapiens], mRNA sequence
Hs.79375	HLCS	holocarboxylase synthetase (biotin-[proprionyl-Coenzyme A-carboxylase (ATP-hydrolysing)] ligase)
Hs.180677	SF1	splicing factor 1
Hs.28785	MFAP3	microfibrillar-associated protein 3
Hs.1437	GAA	glucosidase, alpha; acid (Pompe disease, glycogen storage disease type II)
Hs.77541	ARF5	ADP-ribosylation factor 5
Hs.74376	OLFM1	olfactomedin 1
Hs.75694	MPI	mannose phosphate isomerase
Hs.89232	CBX5	chromobox homolog 5 (HP1 alpha homolog, Drosophila)
Hs.81343	COL2A1	collagen, type II, alpha 1 (primary osteoarthritis, spondyloepiphyseal dysplasia, congenital)
Hs.169963	BTN2A1	butyrophilin, subfamily 2, member A1
Hs.11342	NINJ1	ninjurin 1
Hs.433337	C20orf16	chromosome 20 open reading frame 16
Hs.155553	HNK-1ST	HNK-1 sulfotransferase
Hs.287827	R53330	ESTs, Highly similar to MDR3_HUMAN Multidrug resistance protein 3 (P-glycoprotein 3) [H.sapiens]
Hs.57553	TLK2	tousled-like kinase 2
Hs.2704	GPX2	glutathione peroxidase 2 (gastrointestinal)

Hs.76240	AK1	adenylate kinase 1
Hs.274368	MSTP032	MSTP032 protein
Hs.117950	PAICS	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase
Hs.326	TARBP2	TAR (HIV) RNA binding protein 2
Hs.173125	PPIF	peptidylprolyl isomerase F (cyclophilin F)
Hs.1634	CDC25A	cell division cycle 25A
Hs.106311	DGCR9	DiGeorge syndrome critical region gene 9
Hs.3257	CASP5	caspase 5, apoptosis-related cysteine protease
Hs.75253	IDH3G	isocitrate dehydrogenase 3 (NAD+) gamma
Hs.93728	PBX2	pre-B-cell leukemia transcription factor 2
Hs.388585	AA436278	ESTs, Weakly similar to hypothetical protein FLJ20489 [Homo sapiens] [H.sapiens]
Hs.76297	GPRK6	G protein-coupled receptor kinase 6
Hs.73849	APOC3	apolipoprotein C-III
Hs.433619	PLK	polo-like kinase (Drosophila)
Hs.57419	CTCF	CCCTC-binding factor (zinc finger protein)
Hs.831	HMGCL	3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase (hydroxymethylglutaricaciduria)
Hs.381231	CASP8	caspase 8, apoptosis-related cysteine protease
Hs.106880	BYSL	bystin-like
Hs.278443	FCGR2B	Fc fragment of IgG, low affinity IIb, receptor for (CD32)
Hs.343575	ABI-2	abl-interactor 2
Hs.99881	LDHC	lactate dehydrogenase C
Hs.1872	PCK1	phosphoenolpyruvate carboxykinase 1 (soluble)
Hs.41587	RAD50	RAD50 homolog (S. cerevisiae)
Hs.396489	TF	transferrin
Hs.76781	ABCD3	ATP-binding cassette, sub-family D (ALD), member 3
Hs.49346	SRP54	signal recognition particle 54kDa
Hs.184167	SFRS7	splicing factor, arginine/serine-rich 7, 35kDa
Hs.119475	CIRBP	cold inducible RNA binding protein
Hs.89591	KAL1	Kallmann syndrome 1 sequence
Hs.94382	R12473	EST, Moderately similar to A Chain A, Structure Of Human Adenosine Kinase At 1.50 Angstroms [H.sapiens]
Hs.124027	SPS	selenophosphate synthetase
Hs.2142	HTR3A	5-hydroxytryptamine (serotonin) receptor 3A
Hs.236774	HMGN4	high mobility group nucleosomal binding domain 4
Hs.78977	PCSK1	proprotein convertase subtilisin/kexin type 1
Hs.417218	H08205	EST
Hs.74267	RPL15	ribosomal protein L15
Hs.172740	MAPRE3	microtubule-associated protein, RP/EB family, member 3
Hs.159627	DAP3	death associated protein 3
Hs.153612	ABCF2	ATP-binding cassette, sub-family F (GCN20), member 2
Hs.283016	PDE7B	phosphodiesterase 7B
Hs.6455	RUVBL2	RuvB-like 2 (E. coli)
Hs.252876	MMP23A	matrix metalloproteinase 23A
Hs.125036	TEM7	tumor endothelial marker 7 precursor
Hs.69563	CDC6	CDC6 cell division cycle 6 homolog (S. cerevisiae)
Hs.204354	AA495790	Human HepG2 3' region cDNA, clone hmd1f06, mRNA sequence
Hs.42712	MAX	MAX protein
Hs.7857	EPB41L2	erythrocyte membrane protein band 4.1-like 2
Hs.50282	RAGB	GTP-binding protein ragB
Hs.621	LGALS3	lectin, galactoside-binding, soluble, 3 (galectin 3)
Hs.79348	RGS7	regulator of G-protein signalling 7
Hs.250687	TRPC1	transient receptor potential cation channel, subfamily C, member 1
Hs.388533	R45102	Homo sapiens cDNA FLJ36524 fis, clone TRACH2002960, mRNA sequence
Hs.18069	LGMN	legumain

Hs.378034	W74254	Homo sapiens cDNA FLJ36496 fis, clone THYMU2018819, mRNA sequence
Hs.185923	SLC4A1	solute carrier family 4, anion exchanger, member 1 (erythrocyte membrane protein band 3, Diego blood group)
Hs.433347	AI167344	Homo sapiens cDNA FLJ30687 fis, clone FCBBF2000379, mRNA sequence
Hs.81337	LGALS9	lectin, galactoside-binding, soluble, 9 (galectin 9)
Hs.181015	STAT6	signal transducer and activator of transcription 6, interleukin-4 induced
Hs.155482	HAGH	hydroxyacyl glutathione hydrolase
Hs.171834	PCTK1	PCTAIRE protein kinase 1
Hs.427076	T69468	EST, Moderately similar to RS4Y_HUMAN 40S ribosomal protein S4, Y isoform (PRO2646) [H.sapiens]
Hs.433482	NDUFB7	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7, 18kDa
Hs.80288	HSPA1L	heat shock 70kDa protein 1-like
Hs.2171	GDF10	growth differentiation factor 10
Hs.423615	CDC34	cell division cycle 34
Hs.228249	AI249274	EST
Hs.1846	TP53	tumor protein p53 (Li-Fraumeni syndrome)
Hs.300439	FLJ12089	hypothetical protein FLJ12089
Hs.234234	ALDOB	aldolase B, fructose-bisphosphate
Hs.237356	CXCL12	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)
Hs.75514	NP	nucleoside phosphorylase
Hs.5302	LGALS4	lectin, galactoside-binding, soluble, 4 (galectin 4)
Hs.169517	ALDH1B1	aldehyde dehydrogenase 1 family, member B1
Hs.26401	TNFSF12	tumor necrosis factor (ligand) superfamily, member 12
Hs.426110	AI140253	Homo sapiens cDNA FLJ34678 fis, clone LIVER2003065, mRNA sequence
Hs.179661	AA427899	Beta-tubulin [Homo sapiens], mRNA sequence
Hs.274701	TK2	thymidine kinase 2, mitochondrial
Hs.43834	5'OY11.1	similar to Ovis aries Y chromosome repeat region OY11.1
Hs.274351	ZDHHC9	zinc finger, DHHC domain containing 9
Hs.6980	AKR7A2	aldo-keto reductase family 7, member A2 (aflatoxin aldehyde reductase)
Hs.101850	RBP1	retinol binding protein 1, cellular
Hs.155986	DDX24	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 24
Hs.2131	AVPR1A	arginine vasopressin receptor 1A
Hs.82116	MYD88	myeloid differentiation primary response gene (88)
Hs.407135	AA683578	Homo sapiens mRNA; cDNA DKFZp667G2419 (from clone DKFZp667G2419), mRNA sequence
Hs.2913	EPHB3	EphB3
Hs.378199	GSTA2	glutathione S-transferase A2
Hs.306327	AA039231	Homo sapiens mRNA; cDNA DKFZp434A012 (from clone DKFZp434A012), mRNA sequence
Hs.251653	TUBB2	tubulin, beta, 2
Hs.75251	PIAS1	protein inhibitor of activated STAT, 1
Hs.118836	MB	myoglobin
Hs.380906	LOC91663	hypothetical protein BC013995
Hs.83765	DHFR	dihydrofolate reductase
Hs.74362	CLPP	ClpP caseinolytic protease, ATP-dependent, proteolytic subunit homolog (E. coli)
Hs.247885	HRH2	histamine receptor H2
Hs.211587	PLA2G4A	phospholipase A2, group IVA (cytosolic, calcium-dependent)
Hs.3446	MAP2K1	mitogen-activated protein kinase kinase 1
Hs.348265	CGB1	chorionic gonadotropin, beta polypeptide 1
Hs.355780	R52852	Homo sapiens cDNA FLJ38058 fis, clone CTONG2014898, mRNA sequence
Hs.429898	AA465366	ESTs, Highly similar to LKHA_HUMAN Leukotriene A-4 hydrolase (LTA-4 hydrolase) (Leukotriene A(4) hydrolase) [H.sapiens]

Hs.82614	GYS2	glycogen synthase 2 (liver)
Hs.234799	BCR	breakpoint cluster region
Hs.424980	R55130	ESTs, Highly similar to 5-hydroxytryptamine (serotonin) receptor 2A [Homo sapiens] [H.sapiens]
Hs.14623	IFI30	interferon, gamma-inducible protein 30
Hs.406504	TAGLN2	transgelin 2
Hs.153322	PLCL1	phospholipase C-like 1
Hs.211614	CLCN6	chloride channel 6
Hs.210862	TBR1	T-box, brain, 1
Hs.350378	AA551124	Homo sapiens cDNA FLJ12825 fis, clone NT2RP2002800, mRNA sequence
Hs.75360	CPE	carboxypeptidase E
Hs.430146	AA488168	EST, Highly similar to dynactin 1, isoform 1; dynactin 1 (p150, Glued (Drosophila) homolog); p150, Glued (Drosophila) homolog; 150 kDa dynein-associated polypeptide; p150-glued [Homo sapiens] [H.sapiens]
Hs.73722	APEX1	APEX nuclease (multifunctional DNA repair enzyme) 1
Hs.7879	IFRD1	interferon-related developmental regulator 1
Hs.81972	SHC1	SHC (Src homology 2 domain containing) transforming protein 1
Hs.350321	RET	ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease)
Hs.82208	ACADVL	acyl-Coenzyme A dehydrogenase, very long chain
Hs.355814	T98442	Homo sapiens clone IMAGE:29333, mRNA sequence
Hs.226213	CYP51	cytochrome P450, 51 (lanosterol 14-alpha-demethylase)
Hs.78683	USP7	ubiquitin specific protease 7 (herpes virus-associated)
Hs.355009	T65407	ESTs
Hs.82506	KIAA1254	KIAA1254 protein
Hs.260523	NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog
Hs.153221	SS18	synovial sarcoma translocation, chromosome 18
Hs.75238	CHAF1B	chromatin assembly factor 1, subunit B (p60)
Hs.92261	PDK2	pyruvate dehydrogenase kinase, isoenzyme 2
Hs.8364	PDK4	pyruvate dehydrogenase kinase, isoenzyme 4
Hs.29475	TYMS	thymidylate synthetase
Hs.15432	DOC1	downregulated in ovarian cancer 1
Hs.6755	RPIP8	RaP2 interacting protein 8
Hs.355851	R40790	ESTs, Highly similar to GAC2_HUMAN Gamma-aminobutyric-acid receptor gamma-2 subunit precursor (GABA(A) receptor) [H.sapiens]
Hs.117005	SIGLEC5	sialic acid binding Ig-like lectin 5
Hs.278613	IFI27	interferon, alpha-inducible protein 27
Hs.46362	HTR2C	5-hydroxytryptamine (serotonin) receptor 2C
Hs.147989	CACNG1	calcium channel, voltage-dependent, gamma subunit 1
Hs.709	DCK	deoxycytidine kinase
Hs.279919	RBX1	ring-box 1
Hs.381044	TAF15	TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 68kDa
Hs.284289	VIT1	vitiligo-associated protein VIT-1
Hs.119597	SCD	stearoyl-CoA desaturase (delta-9-desaturase)
Hs.25640	CLDN3	claudin 3
Hs.154868	CAD	carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase
Hs.71622	SMARCD3	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3
Hs.179661	AA427899	Beta-tubulin [Homo sapiens], mRNA sequence
Hs.374535	H85819	Homo sapiens mRNA; cDNA DKFZp434E1835 (from clone DKFZp434E1835), mRNA sequence
Hs.356309	AA676970	ESTs, Highly similar to PMG1_HUMAN Phosphoglycerate mutase 1 (Phosphoglycerate mutase isozyme B) (PGAM-B) (BPG-dependent PGAM 1) [H.sapiens]
Hs.352272	VAV2	vav 2 oncogene

Hs.15243	NOL1	nucleolar protein 1, 120kDa
Hs.1799	CD1D	CD1D antigen, d polypeptide
Hs.54451	LAMC2	laminin, gamma 2
Hs.82001	PKD2	polycystic kidney disease 2 (autosomal dominant)
Hs.118625	HK1	hexokinase 1
Hs.331904	CACNG4	calcium channel, voltage-dependent, gamma subunit 4
Hs.406068	UBE2M	ubiquitin-conjugating enzyme E2M (UBC12 homolog, yeast)
Hs.277704	HYOU1	hypoxia up-regulated 1
Hs.99847	PEX1	peroxisome biogenesis factor 1
Hs.82002	EDNRB	endothelin receptor type B
Hs.75108	RNH	ribonuclease/angiogenin inhibitor
Hs.180878	LPL	lipoprotein lipase
Hs.73999	TRIP10	thyroid hormone receptor interactor 10
Hs.2057	UMPS	uridine monophosphate synthetase (orotate phosphoribosyl transferase and orotidine-5'-decarboxylase)
Hs.337766	RPL18A	ribosomal protein L18a
Hs.174007	VHL	von Hippel-Lindau syndrome
Hs.154036	TSSC3	tumor suppressing subtransferable candidate 3
Hs.89761	ATP5D	ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit
Hs.173464	FKBP8	FK506 binding protein 8, 38kDa
Hs.331803	AI284071	ESTs, Highly similar to A32800 chaperonin GroEL precursor - human [H.sapiens]
Hs.82353	PROCR	protein C receptor, endothelial (EPCR)
Hs.79322	QARS	glutaminyl-tRNA synthetase
Hs.159238	PTPN14	protein tyrosine phosphatase, non-receptor type 14
Hs.155017	NRIP1	nuclear receptor interacting protein 1
Hs.30148	HIPK3	homeodomain interacting protein kinase 3
Hs.79069	AI571464	Homo sapiens mRNA; cDNA DKFZp434B142 (from clone DKFZp434B142), mRNA sequence
Hs.205353	ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1
Hs.851	ITGAE	integrin, alpha E (antigen CD103, human mucosal lymphocyte antigen 1; alpha polypeptide)
Hs.336678	DTNA	dystrobrevin, alpha
Hs.406631	CDC27	cell division cycle 27
Hs.733	EPB42	erythrocyte membrane protein band 4.2
Hs.380465	FLJ00103	cDNA FLJ00103
Hs.279609	MTCH2	mitochondrial carrier homolog 2
Hs.433810	PSME2	proteasome (prosome, macropain) activator subunit 2 (PA28 beta)
Hs.57690	CRYBA4	crystallin, beta A4
Hs.858	RELB	v-rel reticuloendotheliosis viral oncogene homolog B, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3 (avian)
Hs.36508	CHS1	Chediak-Higashi syndrome 1
Hs.132760	G6PT1	glucose-6-phosphatase, transport (glucose-6-phosphate) protein 1
Hs.268545	CACNG2	calcium channel, voltage-dependent, gamma subunit 2
Hs.367762	KRT6A	keratin 6A
Hs.106469	SUPV3L1	suppressor of var1, 3-like 1 (S. cerevisiae)
Hs.74576	GDI1	GDP dissociation inhibitor 1
Hs.597	GOT1	glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1)
Hs.56186	EGFL3	EGF-like-domain, multiple 3
Hs.87465	HEAB	ATP/GTP-binding protein
Hs.241493	AA281731	Similar to phorbol-12-myristate-13-acetate-induced protein 1 [Homo sapiens], mRNA sequence
Hs.376253	TRAP1	heat shock protein 75
Hs.165263	PHC2	polyhomeotic-like 2 (Drosophila)
Hs.89571	RAD52	RAD52 homolog (S. cerevisiae)
Hs.57732	MAPK11	mitogen-activated protein kinase 11

Hs.76366	BAD	BCL2-antagonist of cell death
Hs.80506	SNRPA1	small nuclear ribonucleoprotein polypeptide A'
Hs.30954	PMVK	phosphomevalonate kinase
Hs.3268	HSPA6	heat shock 70kDa protein 6 (HSP70B')
Hs.84131	TARS	threonyl-tRNA synthetase
Hs.150930	XRCC4	X-ray repair complementing defective repair in Chinese hamster cells 4
Hs.259873	ATSV	axonal transport of synaptic vesicles
Hs.171545	HRB	HIV-1 Rev binding protein
Hs.278589	GTF2I	general transcription factor II, i
Hs.82212	CD53	CD53 antigen
Hs.159428	BAX	BCL2-associated X protein
Hs.25954	IL13RA2	interleukin 13 receptor, alpha 2
Hs.111024	SLC25A1	solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1
Hs.2551	ADRB2	adrenergic, beta-2-, receptor, surface
Hs.83765	DHFR	dihydrofolate reductase
Hs.7771	REA	repressor of estrogen receptor activity
Hs.10094	SLC12A4	solute carrier family 12 (potassium/chloride transporters), member 4
Hs.1074	SFTPC	surfactant, pulmonary-associated protein C
Hs.83169	MMP1	matrix metalloproteinase 1 (interstitial collagenase)
Hs.128087	F2R	coagulation factor II (thrombin) receptor
Hs.355214	KRT14	keratin 14 (epidermolysis bullosa simplex, Dowling-Meara, Koebner)
Hs.75375	MDH1	malate dehydrogenase 1, NAD (soluble)
Hs.81134	IL1RN	interleukin 1 receptor antagonist
Hs.289271	CYC1	cytochrome c-1
Hs.7117	H23267	H.sapiens mRNA for 5'UTR for unknown protein (clone ICRFp507O0882), mRNA sequence
Hs.20521	HRMT1L2	HMT1 hnRNP methyltransferase-like 2 (S. cerevisiae)
Hs.372758	UBE2H	ubiquitin-conjugating enzyme E2H (UBC8 homolog, yeast)
Hs.403436	DCI	dodecenoyl-Coenzyme A delta isomerase (3,2 trans-enoyl-Coenzyme A isomerase)
Hs.122116	RUNX2	runt-related transcription factor 2
Hs.73965	SFRS2	splicing factor, arginine/serine-rich 2
Hs.158237	ITGA10	integrin, alpha 10
Hs.239760	CS	citrate synthase
Hs.423329	T65758	Pyruvate dehydrogenase alpha subunit; E1 alpha [Homo sapiens], mRNA sequence
Hs.624	IL8	interleukin 8
Hs.1219	ADH4	alcohol dehydrogenase 4 (class II), pi polypeptide
Hs.76152	AQP1	aquaporin 1 (channel-forming integral protein, 28kDa)
Hs.28408	LTB4R	leukotriene B4 receptor
Hs.10237	ABCG1	ATP-binding cassette, sub-family G (WHITE), member 1
Hs.11689	NOTCH4	Notch homolog 4 (Drosophila)
Hs.48876	FDFT1	farnesyl-diphosphate farnesyltransferase 1
Hs.2994	PCTK3	PCTAIRE protein kinase 3
Hs.78712	ALAS1	aminolevulinate, delta-, synthase 1
Hs.94581	SULT2B1	sulfotransferase family, cytosolic, 2B, member 1
Hs.174140	ACLY	ATP citrate lyase
Hs.432428	PSMA3	proteasome (prosome, macropain) subunit, alpha type, 3
Hs.1861	MPP1	membrane protein, palmitoylated 1, 55kDa
Hs.98243	SPINK2	serine protease inhibitor, Kazal type, 2 (acrosin-trypsin inhibitor)
Hs.249200	FGF19	fibroblast growth factor 19
Hs.82646	DNAJB1	DnaJ (Hsp40) homolog, subfmaily B, member 1
Hs.94785	TGIF2	TGFB-induced factor 2 (TALE family homeobox)
Hs.73987	CLK3	CDC-like kinase 3
Hs.194662	CNN3	calponin 3, acidic
Hs.53250	BMPR2	bone morphogenetic protein receptor, type II (serine/threonine kinase)
Hs.432811	DGUOK	deoxyguanosine kinase

Hs.71465	SQLE	squalene epoxidase
Hs.75932	NAPA	N-ethylmaleimide-sensitive factor attachment protein, alpha
Hs.284202	XPNPEP1	X-prolyl aminopeptidase (aminopeptidase P) 1, soluble
Hs.65993	CDC14A	CDC14 cell division cycle 14 homolog A (S. cerevisiae)
Hs.406386	CLNS1A	chloride channel, nucleotide-sensitive, 1A
Hs.399736	ARL2	ADP-ribosylation factor-like 2
Hs.273415	ALDOA	aldolase A, fructose-bisphosphate
Hs.93213	BAK1	BCL2-antagonist/killer 1
Hs.78040	KDELR1	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 1
Hs.432765	TFAP2A	transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)
Hs.40403	CITED1	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1
Hs.75618	RAB11A	RAB11A, member RAS oncogene family
Hs.433750	EIF4G1	eukaryotic translation initiation factor 4 gamma, 1
Hs.422275	AA425446	ESTs, Highly similar to serine/threonine kinase 14 alpha [Homo sapiens] [H.sapiens]
Hs.46700	ING1	inhibitor of growth family, member 1
Hs.75183	CYP2E1	cytochrome P450, subfamily IIE (ethanol-inducible), polypeptide 1
Hs.406325	PDE7A	phosphodiesterase 7A
Hs.349470	SNCG	synuclein, gamma (breast cancer-specific protein 1)
Hs.9700	CCNE1	cyclin E1
Hs.75372	NAGA	N-acetylgalactosaminidase, alpha-
Hs.80342	KRT15	keratin 15
Hs.62245	SLC25A17	solute carrier family 25 (mitochondrial carrier; peroxisomal membrane protein, 34kDa), member 17
Hs.414985	H56265	ESTs, Highly similar to GSH1_HUMAN Glutamate--cysteine ligase catalytic subunit (Gamma-glutamylcysteine synthetase) (Gamma-ECS) (GCS heavy chain) [H.sapiens]
Hs.73010	IFNW1	interferon, omega 1
Hs.79353	TFDP1	transcription factor Dp-1
Hs.399713	EFNA1	ephrin-A1
Hs.380774	DDX3	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3
Hs.423935	AA056390	Similar to RD RNA-binding protein [Homo sapiens], mRNA sequence
Hs.334562	CDC2	cell division cycle 2, G1 to S and G2 to M
Hs.2707	GSPT1	G1 to S phase transition 1
Hs.1311	CD1C	CD1C antigen, c polypeptide
Hs.38481	CDK6	cyclin-dependent kinase 6
Hs.194143	BRCA1	breast cancer 1, early onset
Hs.75319	RRM2	ribonucleotide reductase M2 polypeptide
Hs.239926	SC4MOL	sterol-C4-methyl oxidase-like
Hs.275775	SEPP1	selenoprotein P, plasma, 1
Hs.1592	CDC16	CDC16 cell division cycle 16 homolog (S. cerevisiae)
Hs.79088	RCN2	reticulocalbin 2, EF-hand calcium binding domain
Hs.321164	NPAS2	neuronal PAS domain protein 2
Hs.1011	PROZ	protein Z, vitamin K-dependent plasma glycoprotein
Hs.110364	PPIC	peptidylprolyl isomerase C (cyclophilin C)
Hs.25960	MYCN	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)
Hs.80350	PPP2CB	protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform
Hs.6650	VPS45A	vacuolar protein sorting 45A (yeast)
Hs.78943	BLMH	bleomycin hydrolase
Hs.180034	CSTF3	cleavage stimulation factor, 3' pre-RNA, subunit 3, 77kDa
Hs.293885	GARS	glycyl-tRNA synthetase
Hs.82269	PAEP	progestagen-associated endometrial protein (placental protein 14, pregnancy-associated endometrial alpha-2-globulin, alpha uterine protein)

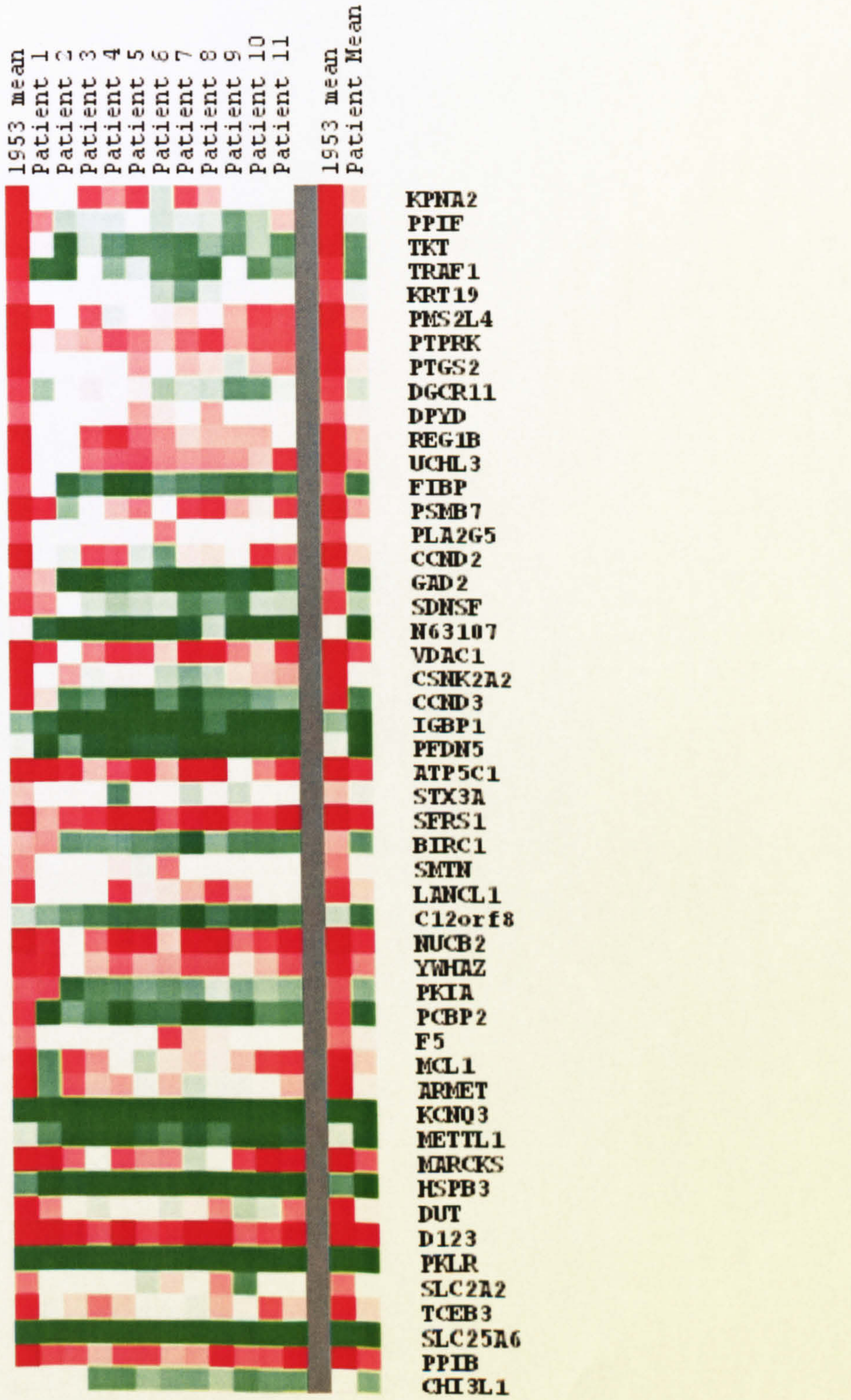
Hs.50002	CCL19	chemokine (C-C motif) ligand 19
Hs.2726	HMGA2	high mobility group AT-hook 2
Hs.110630	W80632	Human BRCA2 region, mRNA sequence CG006
Hs.356081	AA779079	ESTs
Hs.84673	TNNI1	troponin I, skeletal, slow
Hs.129055	ODF2	outer dense fiber of sperm tails 2
Hs.174070	E2-EPF	ubiquitin carrier protein
Hs.2132	EPS8	epidermal growth factor receptor pathway substrate 8
Hs.293007	NPEPPS	aminopeptidase puromycin sensitive
Hs.193124	PDK3	pyruvate dehydrogenase kinase, isoenzyme 3
Hs.4295	PSMD12	proteasome (prosome, macropain) 26S subunit, non-ATPase, 12
Hs.154294	DLG1	discs, large (Drosophila) homolog 1
Hs.279902	CRSP9	cofactor required for Sp1 transcriptional activation, subunit 9, 33kDa
Hs.1697	ATP6V1B2	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B, isoform 2
Hs.115181	ANGPT2	angiopoietin 2
Hs.379781	AA630776	Homo sapiens cDNA FLJ37141 fis, clone BRACE2023801, highly similar to Homo sapiens AP-3 complex delta subunit mRNA, mRNA sequence
Hs.234773	Evi1	ecotropic viral integration site 1
Hs.37009	ALPI	alkaline phosphatase, intestinal
Hs.342874	TGFBR3	transforming growth factor, beta receptor III (betaglycan, 300kDa)
Hs.115474	RFC3	replication factor C (activator 1) 3, 38kDa
Hs.245342	FLJ14642	hypothetical protein FLJ14642
Hs.331602	AP1B1	adaptor-related protein complex 1, beta 1 subunit
Hs.78853	UNG	uracil-DNA glycosylase
Hs.75730	SRPR	signal recognition particle receptor ('docking protein')
Hs.76913	PSMA5	proteasome (prosome, macropain) subunit, alpha type, 5
Hs.159546	ABCD1	ATP-binding cassette, sub-family D (ALD), member 1
Hs.172550	PTBP1	polypyrimidine tract binding protein 1
Hs.169139	KYNU	kynureninase (L-kynurenine hydrolase)
Hs.288642	GNAL	guanine nucleotide binding protein (G protein), alpha activating activity polypeptide, olfactory type
Hs.1189	E2F3	E2F transcription factor 3
Hs.168075	KPNB2	karyopherin (importin) beta 2
Hs.502	TAP2	transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)
Hs.14894	TGOLN2	trans-golgi network protein 2
Hs.71816	AKT1	v-akt murine thymoma viral oncogene homolog 1
Hs.173381	DPYSL2	dihydropyrimidinase-like 2
Hs.25723	SSSCA1	Sjogren's syndrome/scleroderma autoantigen 1
Hs.23582	TACSTD2	tumor-associated calcium signal transducer 2
Hs.51147	GNAT1	guanine nucleotide binding protein (G protein), alpha transducing activity polypeptide 1
Hs.246970	MAP4K5	mitogen-activated protein kinase kinase kinase kinase 5
Hs.101657	AA487543	ESTs, Highly similar to cysteine and histidine-rich domain (CHORD)-containing, zinc-binding protein 1; chord domain-containing protein 1 [Homo sapiens] [H.sapiens]
Hs.1345	MCC	mutated in colorectal cancers
Hs.154443	MCM4	MCM4 minichromosome maintenance deficient 4 (S. cerevisiae)
Hs.2132	EPS8	epidermal growth factor receptor pathway substrate 8
Hs.74631	BSG	basigin (OK blood group)
Hs.24756	HGS	hepatocyte growth factor-regulated tyrosine kinase substrate
Hs.2490	CASP1	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)
Hs.102	AMT	aminomethyltransferase (glycine cleavage system protein T)
Hs.347285	DGCR6L	DiGeorge syndrome critical region gene 6-like
Hs.102482	MUC5B	mucin 5, subtype B, tracheobronchial
Hs.406161	H90348	ESTs, Highly similar to PCL1_HUMAN Prenylcysteine lyase precursor [H.sapiens]

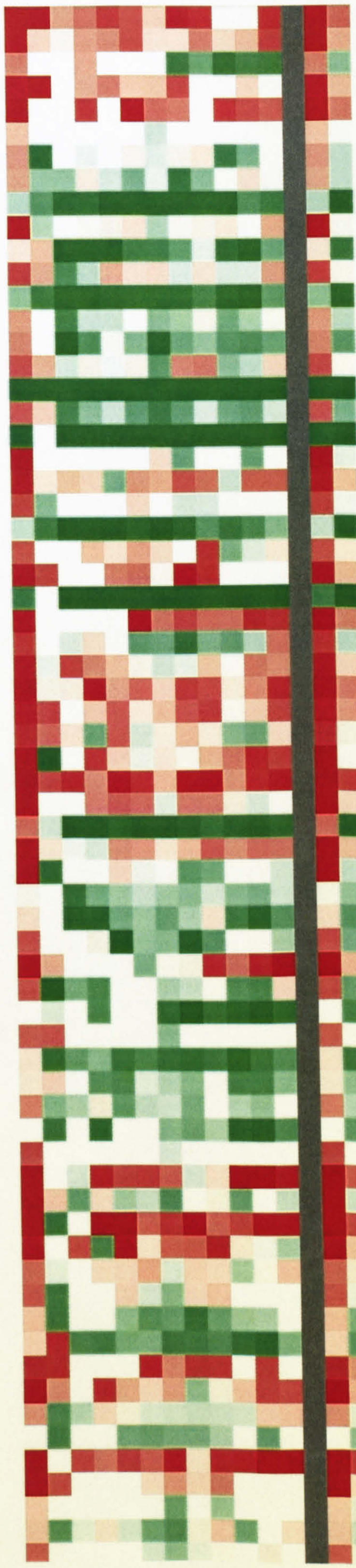
Hs.387463	F8A	coagulation factor VIII-associated (intronic transcript)
Hs.23881	KRT7	keratin 7
Hs.75093	PLOD	procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase, Ehlers-Danlos syndrome type VI)
Hs.126256	IL1B	interleukin 1, beta
Hs.335918	FDPS	farnesyl diphosphate synthase (farnesyl pyrophosphate synthetase, dimethylallyltranstransferase, geranyltranstransferase)
Hs.2795	LDHA	lactate dehydrogenase A
Hs.281866	ATP6V1A1	ATPase, H+ transporting, lysosomal 70kDa, V1 subunit A, isoform 1
Hs.153591	NOT56L	Not56 (D. melanogaster)-like protein
Hs.183800	RANGAP1	Ran GTPase activating protein 1
Hs.239298	MAP4	microtubule-associated protein 4
Hs.153684	FRZB	frizzled-related protein
Hs.6631	FLJ20373	hypothetical protein FLJ20373
Hs.153937	ACK1	activated p21cdc42Hs kinase
Hs.337616	PDE3B	phosphodiesterase 3B, cGMP-inhibited
Hs.159553	CMKLR1	chemokine-like receptor 1
Hs.118825	MAP2K6	mitogen-activated protein kinase kinase 6
Hs.433955	FLJ20886	hypothetical protein FLJ20886
Hs.861	MAPK3	mitogen-activated protein kinase 3
Hs.673	IL12A	interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)
Hs.75593	UROS	uroporphyrinogen III synthase (congenital erythropoietic porphyria)
Hs.10526	CSRP2	cysteine and glycine-rich protein 2
Hs.93659	ERP70	protein disulfide isomerase related protein (calcium-binding protein, intestinal-related)
Hs.1817	MPO	myeloperoxidase
Hs.72241	MAP2K2	mitogen-activated protein kinase kinase 2
Hs.84746	CHC1	chromosome condensation 1
Hs.35120	RFC4	replication factor C (activator 1) 4, 37kDa
Hs.86978	PREP	prolyl endopeptidase
Hs.82927	AMPD2	adenosine monophosphate deaminase 2 (isoform L)
Hs.8257	CISH	cytokine inducible SH2-containing protein
Hs.80776	PLCD1	phospholipase C, delta 1
Hs.20084	RXRA	retinoid X receptor, alpha
Hs.310512	CCRL1	chemokine (C-C motif) receptor-like 1
Hs.153752	CDC25B	cell division cycle 25B
Hs.26886	CLUL1	clusterin-like 1 (retinal)
Hs.169793	RPL32	ribosomal protein L32
Hs.8724	STK38	serine/threonine kinase 38
Hs.355719	NPM1	nucleophosmin (nucleolar phosphoprotein B23, numatrin)
Hs.172108	NUP88	nucleoporin 88kDa
Hs.433389	KRT18L1	keratin 18-like 1
Hs.81217	FZD2	frizzled homolog 2 (Drosophila)
Hs.78465	JUN	v-jun sarcoma virus 17 oncogene homolog (avian)
Hs.21486	STAT1	signal transducer and activator of transcription 1, 91kDa
Hs.136502	AA021598	ESTs
Hs.291904	BCAP31	accessory protein BAP31
Hs.301449	BS69	adenovirus 5 E1A binding protein
Hs.252723	RPL19	ribosomal protein L19
Hs.76536	TBL1X	transducin (beta)-like 1X-linked
Hs.1565	NEDD4	neural precursor cell expressed, developmentally down-regulated 4
Hs.75244	BCL2L2	BCL2-like 2
Hs.740	PTK2	PTK2 protein tyrosine kinase 2
Hs.20768	HSPC189	HSPC189 protein
Hs.374491	PA2G4	proliferation-associated 2G4, 38kDa
Hs.381081	PSMB9	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional protease 2)

Hs.73980	TNNT1	troponin T1, skeletal, slow
Hs.334345	CYP2A6	cytochrome P450, subfamily IIA (phenobarbital-inducible), polypeptide 6
Hs.724	THRA	thyroid hormone receptor, alpha (erythroblastic leukemia viral (v-erb-a) oncogene homolog, avian)
Hs.198760	NEFH	neurofilament, heavy polypeptide 200kDa
Hs.27076	RTCD1	RTC domain containing 1
Hs.68137	ADCYAP1	adenylate cyclase activating polypeptide 1 (pituitary)
Hs.2025	TGFB3	transforming growth factor, beta 3
Hs.197366	SMOH	smoothened homolog (Drosophila)
Hs.196352	NCF4	neutrophil cytosolic factor 4, 40kDa
Hs.11951	ENPP1	ectonucleotide pyrophosphatase/phosphodiesterase 1
Hs.250666	HRY	hairy homolog (Drosophila)
Hs.431288	R23773	ESTs, Moderately similar to melanoma antigen, family A, 10 [Homo sapiens] [H.sapiens]
Hs.356739	PPP6C	protein phosphatase 6, catalytic subunit
Hs.95998	FRDA	Friedreich ataxia
Hs.100980	PPM1D	protein phosphatase 1D magnesium-dependent, delta isoform
Hs.84981	XRCC5	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining; Ku autoantigen, 80kDa)
Hs.86905	ATP6V1C1	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C, isoform 1
Hs.279906	CCNT1	cyclin T1
Hs.11393	RAD51C	RAD51 homolog C (S. cerevisiae)
Hs.74034	CAV1	caveolin 1, caveolae protein, 22kDa
Hs.89563	NCBP1	nuclear cap binding protein subunit 1, 80kDa
Hs.1216	ACTN3	actinin, alpha 3
Hs.433523	KIAA0864	KIAA0864 protein
Hs.7753	CALU	calumenin
Hs.26014	ACVR2	activin A receptor, type II
Hs.84264	ANP32B	acidic (leucine-rich) nuclear phosphoprotein 32 family, member B
Hs.25274	C11ORF4	chromosome 11 hypothetical protein ORF4
Hs.73165	IL12RB2	interleukin 12 receptor, beta 2
Hs.173311	C18B11	C18B11 homolog (44.9kD)
Hs.2903	PPP4C	protein phosphatase 4 (formerly X), catalytic subunit
Hs.177556	MAGED1	melanoma antigen, family D, 1
Hs.173936	IL10RB	interleukin 10 receptor, beta
Hs.160483	STOM	stomatin
Hs.274376	AMY1A	amylase, alpha 1A; salivary
Hs.75981	USP14	ubiquitin specific protease 14 (tRNA-guanine transglycosylase)
Hs.127799	BIRC3	baculoviral IAP repeat-containing 3
Hs.18573	ACYP1	acylphosphatase 1, erythrocyte (common) type
Hs.326709	AA867984	Similar to phospholipase A2, group IB (pancreas) [Homo sapiens], mRNA sequence
Hs.2934	RRM1	ribonucleotide reductase M1 polypeptide
Hs.432970	CCT2	chaperonin containing TCP1, subunit 2 (beta)
Hs.171695	DUSP1	dual specificity phosphatase 1
Hs.75741	ABP1	amiloride binding protein 1 (amine oxidase (copper-containing))
Hs.296341	CAP2	adenylyl cyclase-associated protein 2
Hs.8700	DLC1	deleted in liver cancer 1
Hs.29117	PURA	purine-rich element binding protein A
Hs.433267	MGC2198	hypothetical protein MGC2198
Hs.181107	ANXA13	annexin A13
Hs.283690	H41	hypothetical protein H41
Hs.347527	SLC20A2	solute carrier family 20 (phosphate transporter), member 2
Hs.55173	CELSR3	cadherin, EGF LAG seven-pass G-type receptor 3 (flamingo homolog, Drosophila)
Hs.227817	BCL2A1	BCL2-related protein A1
Hs.75653	FH	fumarate hydratase

Hs.63668	TLR2	toll-like receptor 2
Hs.101408	BCAT2	branched chain aminotransferase 2, mitochondrial
Hs.343809	FGF12	fibroblast growth factor 12
Hs.94925	DHODH	dihydroorotate dehydrogenase
Hs.40411	AA115310	ESTs, Highly similar to S55507 protein disulfide-isomerase (EC 5.3.4.1) ER60 precursor - human [H.sapiens]
Hs.379960	AA504710	Hypothetical protein [Homo sapiens], mRNA sequence
Hs.904	AGL	amylo-1, 6-glucosidase, 4-alpha-glucanotransferase (glycogen debranching enzyme, glycogen storage disease type III)
Hs.374366	AA486417	Homo sapiens cDNA FLJ37354 fis, clone BRAMY2022929, mRNA sequence
Hs.399762	E2F5	E2F transcription factor 5, p130-binding
Hs.294101	PBX3	pre-B-cell leukemia transcription factor 3
Hs.3136	PRKAG1	protein kinase, AMP-activated, gamma 1 non-catalytic subunit
Hs.167013	DNM2	dynamin 2
Hs.673	IL12A	interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)
Hs.10734	CHRNA4	cholinergic receptor, nicotinic, alpha polypeptide 4
Hs.400275	AA010777	ESTs, Moderately similar to A Chain A, Crystal Structure Of Human Galectin-7 In Complex With Galactose [H.sapiens]
Hs.194778	IL8RA	interleukin 8 receptor, alpha
Hs.321709	P2RX4	purinergic receptor P2X, ligand-gated ion channel, 4
Hs.6241	PIK3R1	phosphoinositide-3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)
Hs.150557	BTEB1	basic transcription element binding protein 1
Hs.119684	TNFRSF10C	tumor necrosis factor receptor superfamily, member 10c, decoy without an intracellular domain
Hs.78743	ZNF131	zinc finger protein 131 (clone pHZ-10)
Hs.9795	ACOX2	acyl-Coenzyme A oxidase 2, branched chain
Hs.19368	MATN2	matrilin 2
Hs.135626	CMA1	chymase 1, mast cell
Hs.159263	COL6A2	collagen, type VI, alpha 2
Hs.234569	ZAP70	zeta-chain (TCR) associated protein kinase 70kDa
Hs.24030	SLC31A2	solute carrier family 31 (copper transporters), member 2
Hs.19261	DYT1	dystonia 1, torsion (autosomal dominant; torsin A)

2.2 Genes with significantly decreased polysome association in B-CLL cells when compared to GM1953 cells. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated.




KARS
TUBB5
LNPEP
IGFBP2
TM4SF1
PSCD3
LIG1
N62761
DTR
BAK1
FUCA1
POR
MAPK4
EREG
SLC1A5
PIP5K1C
DKFZp564A176
GSTM4
ASS
SLC6A12
PCK2
B7H2
PTHR1
SPP1
BAT3
MITF
ALDH1A1
TFRC
TGFBI
NFKB2
BCKDHA
UQCRFS1
CRYAB
SDHB
CPA2
PPP3CC
MRPL19
BRAF
PTGS1
NOC4
ILF1
GPS1
SLC16A1
DNAJC4
BG230619
AA017199
MIPEP
RRAD
RAD51L1
PTGES
HSPCB
TXNL
TNIP2
HNRPK
FGF1
BARD1
GOLGA5
UGT2B4
BRE
LOC51125
MYB
CDK2
KRT5
INSR
SERPINA1
IDI1
CDSN


PRC1
PRKDC
MAPK10
FECH
CD44
DNAJC4
CCK
GTPBP1
POR
AI363781
HNRPM
BLVRA
MSH2
IFRG28
VCAM1
GPC5
PTPN11
H29256
AA188155
T50699
NDUFA9
PRKCI
POLR2D
RPL35
MMP15
MAN1A1
CRYGC
PSME3
CDC7L1
MAP2K6
JTV1
LCK
TRAF1
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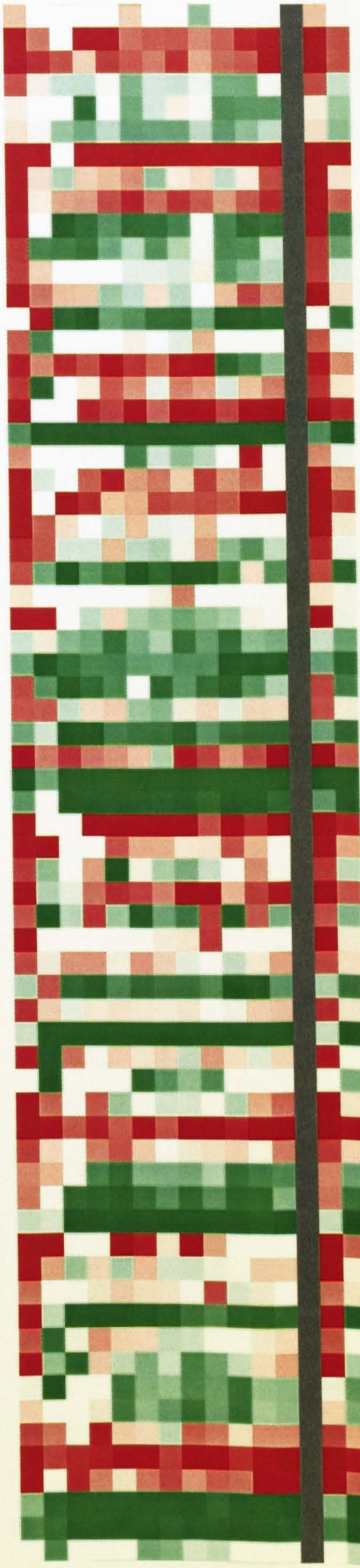

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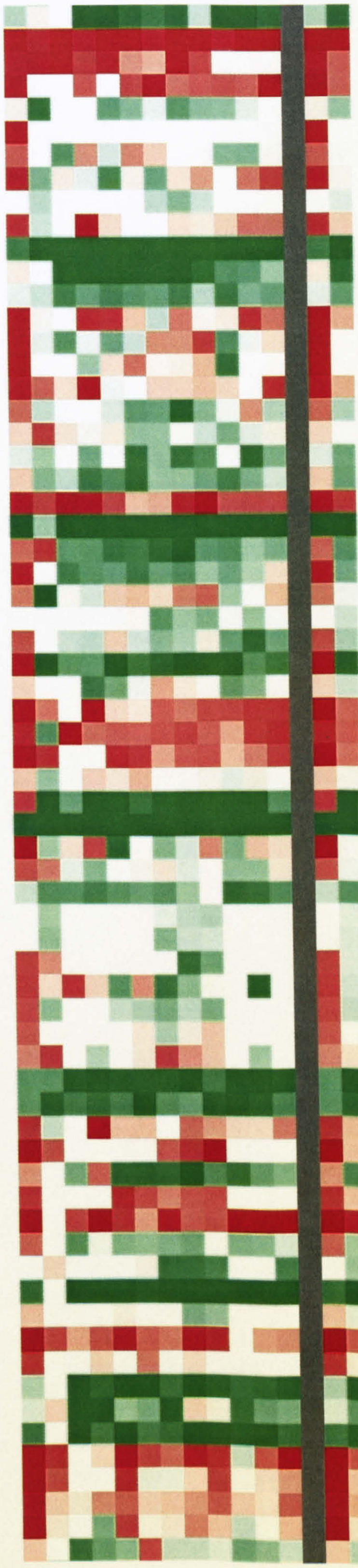

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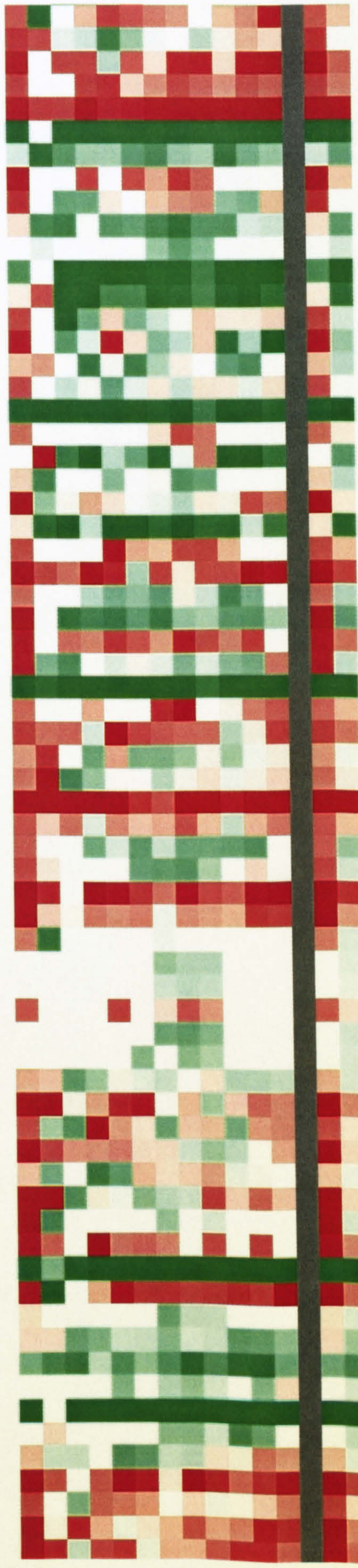

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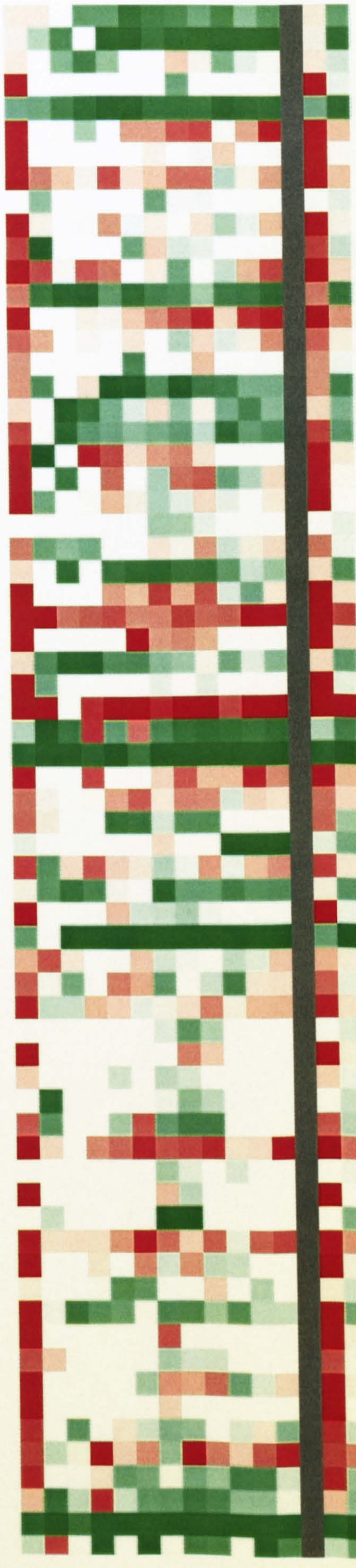

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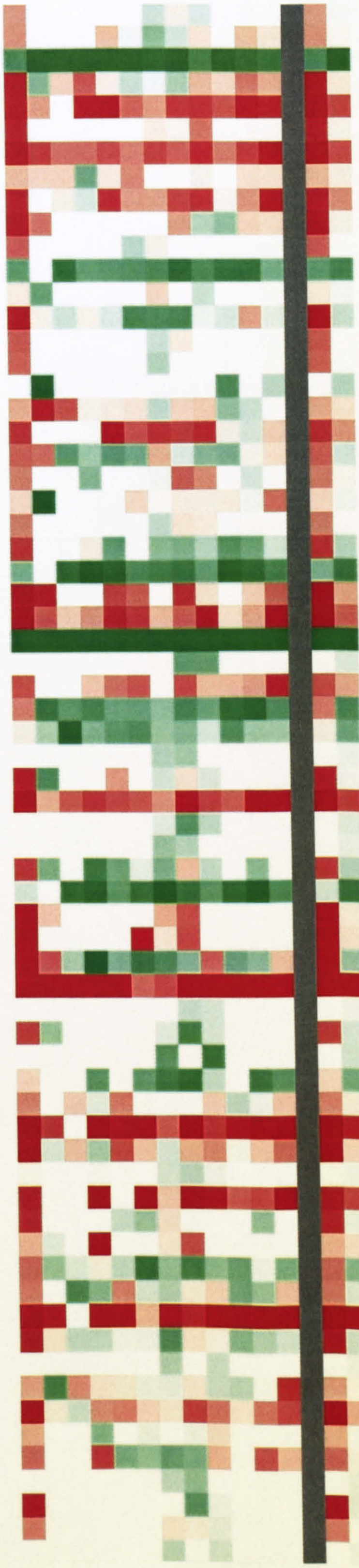

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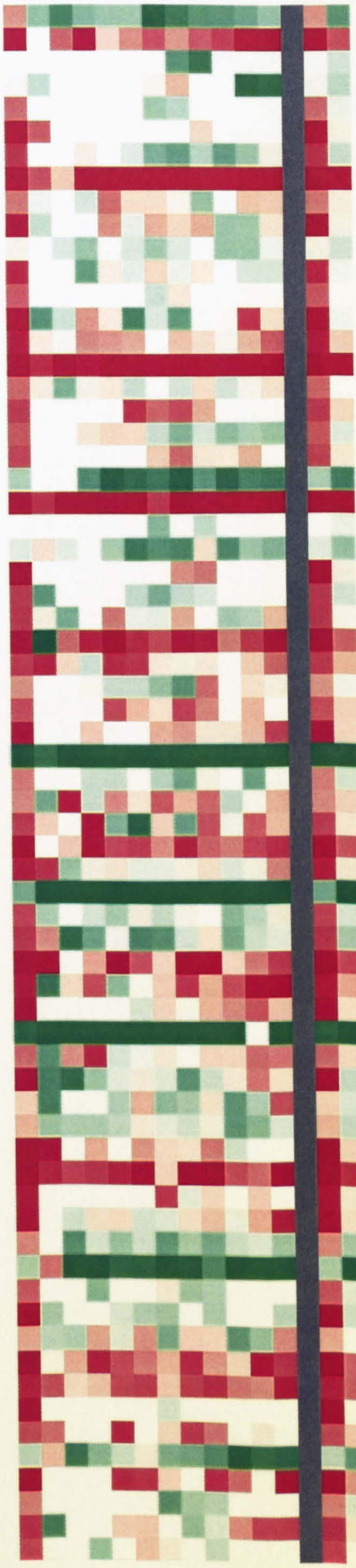

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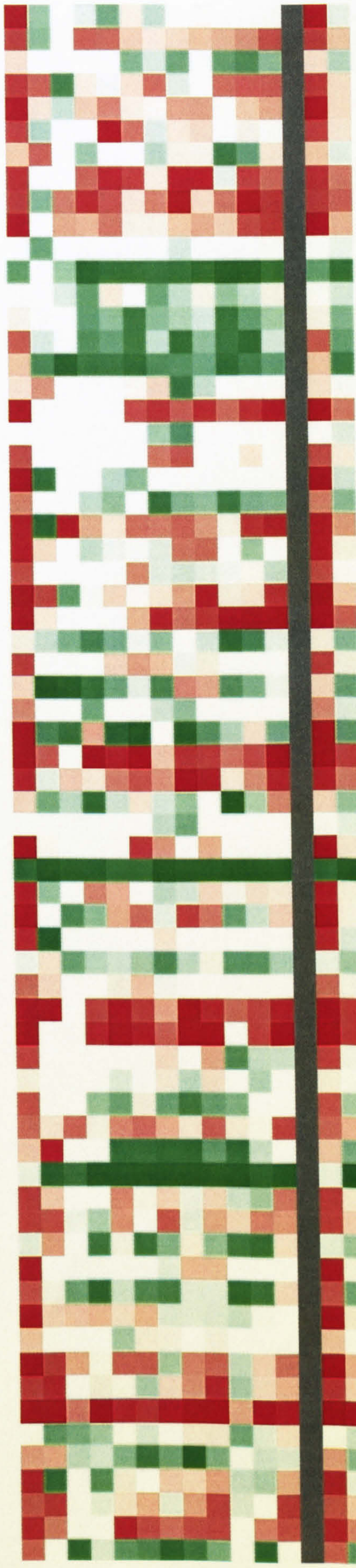
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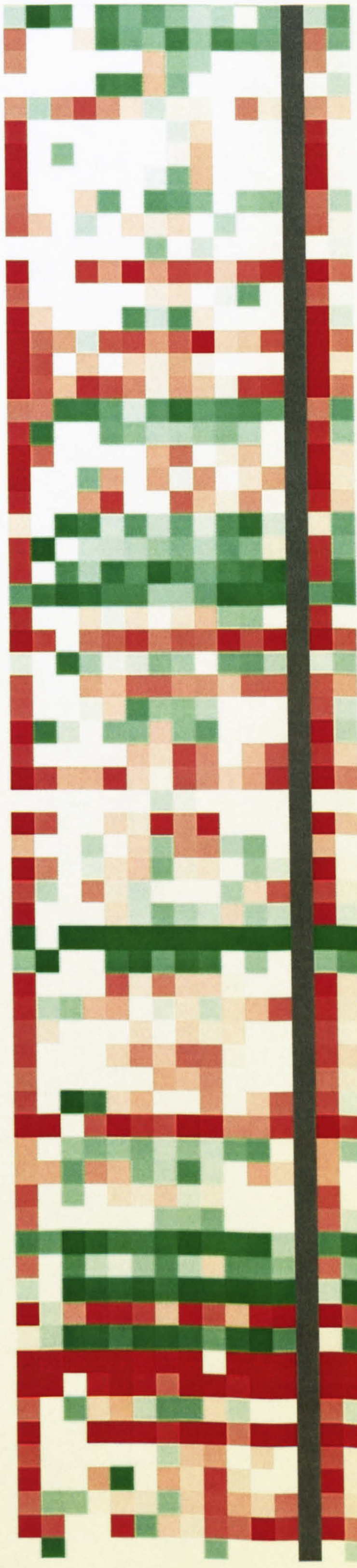
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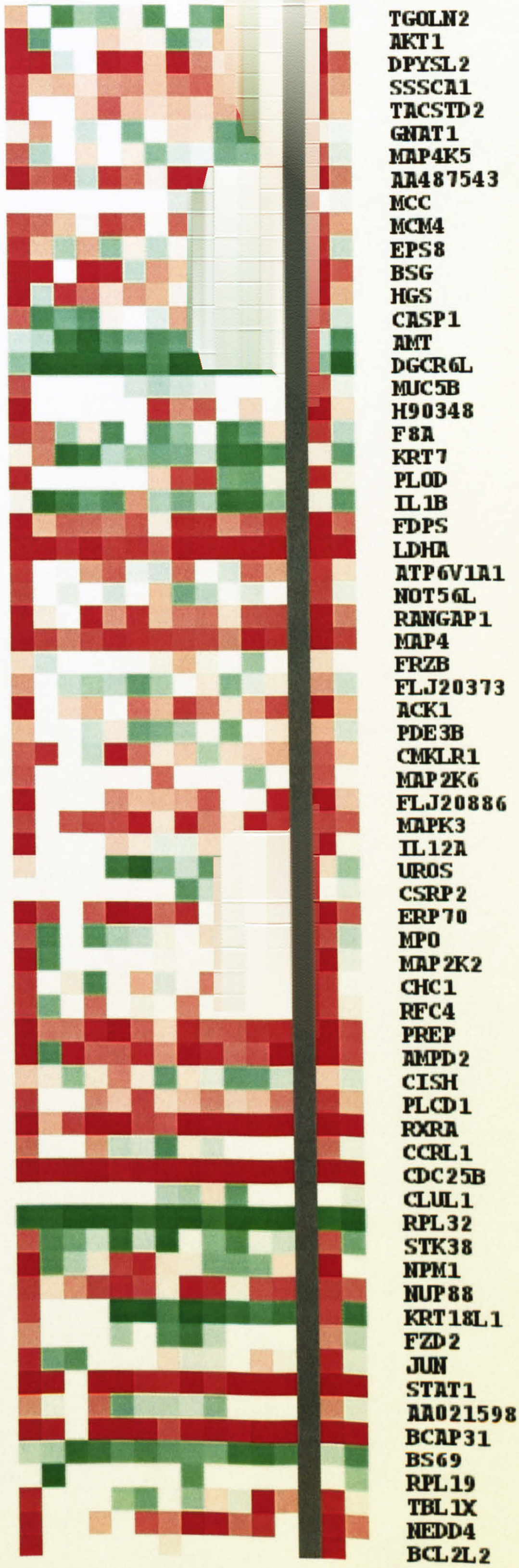
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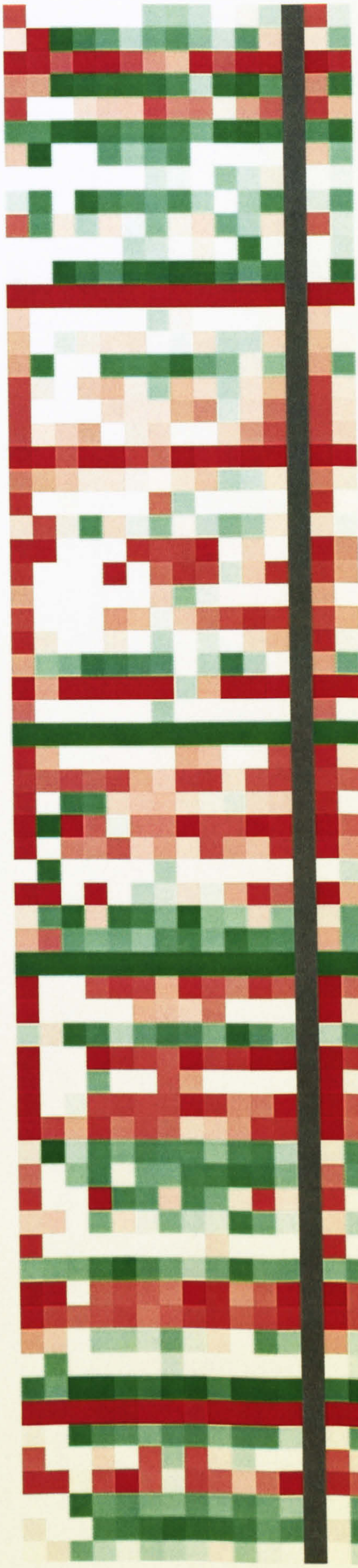

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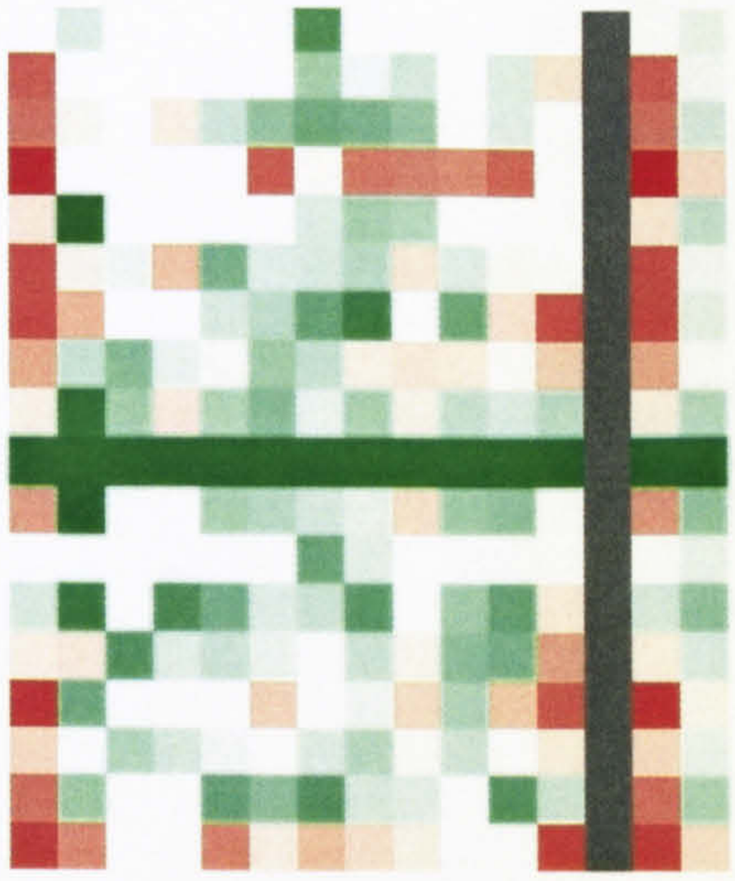

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PRDX4

Appendix 3

3.1 Table of genes with significantly increased polysome association in B-CLL cells when compared to tonsil CD19+ B cells.

Cluster Number	Gene Name	Gene Description
Hs.17165	RGS13	regulator of G-protein signalling 13
Hs.80658	UCP2	uncoupling protein 2 (mitochondrial, proton carrier)
Hs.367762	KRT6A	keratin 6A
Hs.80423	PBP	prostatic binding protein
Hs.356386	RAB7	RAB7, member RAS oncogene family
Hs.11101	DLG3	discs, large (Drosophila) homolog 3 (neuroendocrine-dlg)
Hs.89472	AGTR1	angiotensin II receptor, type 1
Hs.89414	CXCR4	chemokine (C-X-C motif) receptor 4
Hs.85769	HSU15552	acidic 82 kDa protein mRNA
Hs.95327	CD3D	CD3D antigen, delta polypeptide (TiT3 complex)
Hs.388392	R45428	ESTs
Hs.94376	PCSK5	proprotein convertase subtilisin/kexin type 5
Hs.315463	IL24	interleukin 24
Hs.84	IL2RG	interleukin 2 receptor, gamma (severe combined immunodeficiency)
Hs.78589	SERPINI1	serine (or cysteine) proteinase inhibitor, clade I (neuroserpin), member 1
Hs.77054	BTG1	B-cell translocation gene 1, anti-proliferative
Hs.75819	GPM6A	glycoprotein M6A
Hs.9736	PSMD3	proteasome (prosome, macropain) 26S subunit, non-ATPase, 3
Hs.850	IMPDH1	IMP (inosine monophosphate) dehydrogenase 1
Hs.78353	SRPK2	SFRS protein kinase 2
Hs.95577	CDK4	cyclin-dependent kinase 4
Hs.184771	U5-100K	prp28, U5 snRNP 100 kd protein
Hs.236030	SMARCC2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2
Hs.11171	APG5L	APG5 autophagy 5-like (S. cerevisiae)
Hs.27910	CEP2	centrosomal protein 2
Hs.288319	SART1	squamous cell carcinoma antigen recognised by T cells
Hs.35140	STK4	serine/threonine kinase 4
Hs.343586	ZFP36	zinc finger protein 36, C3H type, homolog (mouse)
Hs.77202	PRKCB1	protein kinase C, beta 1
Hs.258503	CYFIP2	cytoplasmic FMR1 interacting protein 2
Hs.78877	ITPKB	inositol 1,4,5-trisphosphate 3-kinase B
Hs.75774	THBS4	thrombospondin 4
Hs.82210	ZNF220	zinc finger protein 220
Hs.856	IFNG	interferon, gamma
Hs.80887	LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog
Hs.1674	GFPT1	glutamine-fructose-6-phosphate transaminase 1
Hs.144063	SARS	seryl-tRNA synthetase
Hs.85226	LIPA	lipase A, lysosomal acid, cholesterol esterase (Wolman disease)
Hs.232400	HNRPA2B1	heterogeneous nuclear ribonucleoprotein A2/B1
Hs.165843	CSNK2B	casein kinase 2, beta polypeptide
Hs.2554	SIAT1	sialyltransferase 1 (beta-galactoside alpha-2,6-sialytransferase)
Hs.183773	GOLGA4	golgi autoantigen, golgin subfamily a, 4
Hs.183684	EIF4G2	eukaryotic translation initiation factor 4 gamma, 2
Hs.300711	ANXA5	annexin A5
Hs.1790	NR3C2	nuclear receptor subfamily 3, group C, member 2
Hs.28785	MFAP3	microfibrillar-associated protein 3

Hs.79008	SNW1	SKI-interacting protein
Hs.315463	IL24	interleukin 24
Hs.343877	CGI-85	CGI-85 protein
Hs.155455	PFKL	phosphofructokinase, liver
Hs.814	HLA-DPB1	major histocompatibility complex, class II, DP beta 1
Hs.1540	THOC1	THO complex 1
Hs.410488	R39356	EST, Weakly similar to hypothetical protein FLJ20489 [Homo sapiens] [H.sapiens]
Hs.277477	HLA-C	major histocompatibility complex, class I, C
Hs.181244	HLA-A	major histocompatibility complex, class I, A
Hs.2910	PRPS2	phosphoribosyl pyrophosphate synthetase 2
Hs.75584	PMSCL2	polymyositis/scleroderma autoantigen 2, 100kDa
Hs.77196	SPTAN1	spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)
Hs.13501	PES1	pescadillo homolog 1, containing BRCT domain (zebrafish)
Hs.288658	ZNF35	zinc finger protein 35 (clone HF.10)
Hs.78344	MYH11	myosin, heavy polypeptide 11, smooth muscle
Hs.374466	WARS	tryptophanyl-tRNA synthetase
Hs.173902	PPP2R1A	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), alpha isoform
Hs.3069	HSPA9B	heat shock 70kDa protein 9B (mortalin-2)
Hs.81008	FLNB	filamin B, beta (actin binding protein 278)
Hs.374973	PRPF4	PRP4 pre-mRNA processing factor 4 homolog (yeast)
Hs.840	INDO	indoleamine-pyrrole 2,3 dioxygenase
Hs.89421	CIR	CBF1 interacting corepressor
Hs.82327	GSS	glutathione synthetase
Hs.144700	EFNB1	ephrin-B1
Hs.25647	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog
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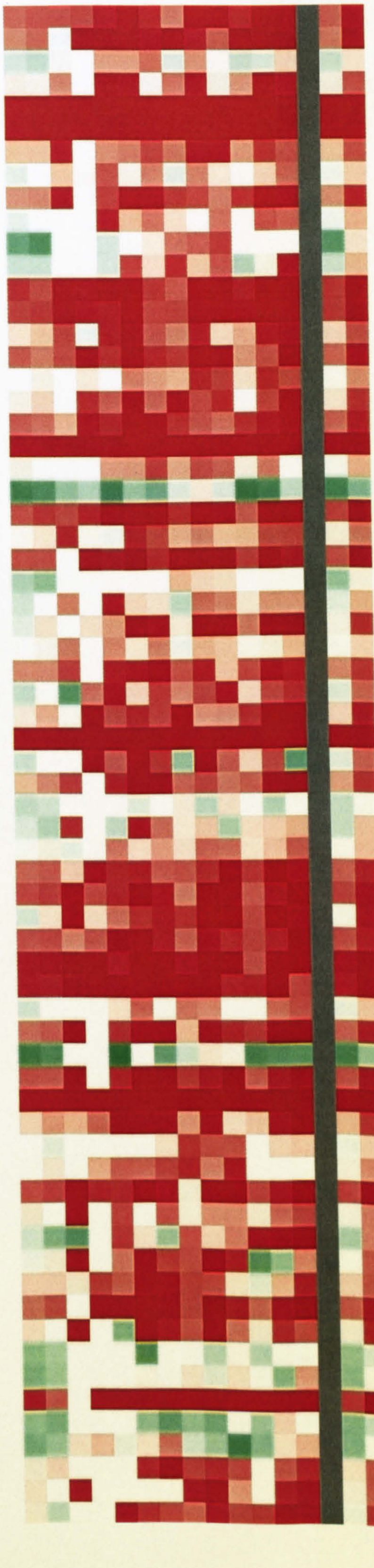
Hs.123078	TSHR	thyroid stimulating hormone receptor
Hs.174071	GYG	glycogenin
Hs.74405	YWHAQ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide
Hs.12409	SST	somatostatin
Hs.38125	SP110	SP110 nuclear body protein
Hs.78629	ATP1B1	ATPase, Na+/K+ transporting, beta 1 polypeptide
Hs.349110	MST1	macrophage stimulating 1 (hepatocyte growth factor-like)
Hs.375570	HLA-DRB1	major histocompatibility complex, class II, DR beta 1
Hs.21016	COCH	coagulation factor C homolog, cochlin (Limulus polyphemus)
Hs.380986	MGC16723	hypothetical protein MGC16723
Hs.98243	SPINK2	serine protease inhibitor, Kazal type, 2 (acrosin-trypsin inhibitor)
Hs.159553	CMKLR1	chemokine-like receptor 1
Hs.79241	BCL2	B-cell CLL/lymphoma 2
Hs.79411	RPA2	replication protein A2, 32kDa
Hs.286055	CHN2	chimerin (chimaerin) 2
Hs.202833	HMOX1	heme oxygenase (decycling) 1
Hs.237825	SRP72	signal recognition particle 72kDa
Hs.77492	HNRPA0	heterogeneous nuclear ribonucleoprotein A0
Hs.82848	SELL	selectin L (lymphocyte adhesion molecule 1)
Hs.75188	WEE1	WEE1 homolog (S. pombe)
Hs.6551	ATP6IP1	ATPase, H+ transporting, lysosomal interacting protein 1
Hs.79391	HD	huntingtin (Huntington disease)
Hs.75140	LRPAP1	low density lipoprotein-related protein-associated protein 1 (alpha-2-macroglobulin receptor-associated protein 1)
Hs.377755	AA634006	Unnamed protein product [Homo sapiens], mRNA sequence
Hs.252280	ARHGEF1	Rho guanine nucleotide exchange factor (GEF) 1

Hs.411904	R55046	EST
Hs.748	FGFR1	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)
Hs.406510	ATP5B	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide
Hs.155206	STK25	serine/threonine kinase 25 (STE20 homolog, yeast)
Hs.169449	PRKCA	protein kinase C, alpha
Hs.76549	ATP1A1	ATPase, Na+/K+ transporting, alpha 1 polypeptide
Hs.75682	RCD-8	autoantigen
Hs.103042	MAP1B	microtubule-associated protein 1B
Hs.174044	DVL3	dishevelled, dsh homolog 3 (Drosophila)
Hs.82240	STX3A	syntaxin 3A
Hs.23119	CXorf12	chromosome X open reading frame 12
Hs.75576	PLG	plasminogen
Hs.89578	GTF2H1	general transcription factor IIH, polypeptide 1, 62kDa
Hs.13512	ZW10	ZW10 homolog, centromere/kinetochore protein (Drosophila)
Hs.8657	PCQAP	PC2 (positive cofactor 2, multiprotein complex) glutamine/Q-rich-associated protein
Hs.3352	HDAC2	histone deacetylase 2
Hs.75741	ABP1	amiloride binding protein 1 (amine oxidase (copper-containing))
Hs.179565	MCM3	MCM3 minichromosome maintenance deficient 3 (S. cerevisiae)
Hs.172199	ADCY7	adenylate cyclase 7
Hs.3657	ADNP	activity-dependent neuroprotector
Hs.155191	VIL2	villin 2 (ezrin)
Hs.12272	BECN1	beclin 1 (coiled-coil, myosin-like BCL2 interacting protein)
Hs.31439	SPINT2	serine protease inhibitor, Kunitz type, 2
Hs.83347	AAMP	angio-associated, migratory cell protein
Hs.423935	AA056390	Similar to RD RNA-binding protein [Homo sapiens], mRNA sequence
Hs.297753	VIM	vimentin
Hs.153261	IGHM	immunoglobulin heavy constant mu
Hs.406423	SF3B2	splicing factor 3b, subunit 2, 145kDa
Hs.83656	ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta
Hs.337766	RPL18A	ribosomal protein L18a
Hs.1741	ITGB7	integrin, beta 7
Hs.75772	NR3C1	nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
Hs.118721	NEU1	sialidase 1 (lysosomal sialidase)
Hs.350899	AA102454	Homo sapiens cDNA FLJ30484 fis, clone BRAWH2000071, mRNA sequence
Hs.433369	AA284856	Human fibroblast mRNA fragment with Alu sequence (pRHF11), mRNA sequence
Hs.89695	INSR	insulin receptor
Hs.376459	AA281945	Homo sapiens cDNA FLJ36300 fis, clone THYMU2004410, mRNA sequence
Hs.118638	NME1	non-metastatic cells 1, protein (NM23A) expressed in
Hs.386741	ANXA7	annexin A7
Hs.211573	HSPG2	heparan sulfate proteoglycan 2 (perlecan)
Hs.1279	C1R	complement component 1, r subcomponent
Hs.84318	RPA1	replication protein A1, 70kDa
Hs.1298	MME	membrane metallo-endopeptidase (neutral endopeptidase, enkephalinase, CALLA, CD10)
Hs.288433	HNT	neurotrimin
Hs.7957	ADAR	adenosine deaminase, RNA-specific
Hs.79351	KCNK1	potassium channel, subfamily K, member 1
Hs.171880	POLR2A	polymerase (RNA) II (DNA directed) polypeptide A, 220kDa
Hs.56937	ST14	suppression of tumorigenicity 14 (colon carcinoma, matriptase, epithin)

Hs.93213	BAK1	BCL2-antagonist/killer 1
Hs.178658	RAD23B	RAD23 homolog B (S. cerevisiae)
Hs.211582	MYLK	myosin, light polypeptide kinase
Hs.75450	DSIPI	delta sleep inducing peptide, immunoreactor
Hs.240457	RAD9	RAD9 homolog (S. pombe)
Hs.75149	SH3GL2	SH3-domain GRB2-like 2
Hs.118962	FUBP1	far upstream element (FUSE) binding protein 1
Hs.89781	UBTF	upstream binding transcription factor, RNA polymerase I
Hs.21486	STAT1	signal transducer and activator of transcription 1, 91kDa
Hs.180877	H3F3B	H3 histone, family 3B (H3.3B)
Hs.6335	MGC45562	hypothetical protein MGC45562
Hs.198296	SMARCA2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2
Hs.6430	CHERP	calcium homeostasis endoplasmic reticulum protein
Hs.273307	SRP68	signal recognition particle 68kDa
Hs.277477	HLA-C	major histocompatibility complex, class I, C
Hs.85302	ADARB1	adenosine deaminase, RNA-specific, B1 (RED1 homolog rat)
Hs.112028	MINK	misshapen/NIK-related kinase
Hs.469	SDHA	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)
Hs.326248	PDCD4	programmed cell death 4 (neoplastic transformation inhibitor)
Hs.20478	CLN2	ceroid-lipofuscinosis, neuronal 2, late infantile (Jansky-Bielschowsky disease)
Hs.349530	YWHAH	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide
Hs.431496	AA775803	EST, Moderately similar to I68897 probable thioredoxin peroxidase (EC 1.11.1.-) 1 - human [H.sapiens]
Hs.63668	TLR2	toll-like receptor 2
Hs.78771	PGK1	phosphoglycerate kinase 1
Hs.73923	PNLIPRP1	pancreatic lipase-related protein 1
Hs.33642	ARCN1	archain 1
Hs.41296	FLRT3	fibronectin leucine rich transmembrane protein 3
Hs.75811	ASAH1	N-acylsphingosine amidohydrolase (acid ceramidase) 1
Hs.37860	KLF1	Kruppel-like factor 1 (erythroid)
Hs.79064	DHPS	deoxyhypusine synthase
Hs.256747	SPAG8	sperm associated antigen 8
Hs.343564	TBCE	tubulin-specific chaperone e
Hs.197345	G22P1	thyroid autoantigen 70kDa (Ku antigen)
Hs.17165	RGS13	regulator of G-protein signalling 13

3.2 Genes with significantly increased polysome association in B-CLL cells when compared to tonsil CD19+ B cells. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated.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SPTAN1
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WARS
PPP2R1A
HSPA9B
FLNB
PRPF4
INDO
CIR
GSS
EFNB1
FOS
IFI16
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NEDD5
TSHR
GYG
YWHAQ
SST
SP110
ATP1B1
MST1
HLA-DRB1
COCH
MGC16723
SPINK2
CMKLR1
BCL2
RPA2
CHN2
HMOX1
SRP72
HNRPA0
SELL
WEE1
ATP6IP1
HD
LRPAP1
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ARHGEF1
R55046
FGFR1
ATP5B
STK25
PRKCA
ATP1A1
RCD-8
MAP1B
DVL3
STX3A
CXorf12
PLG
GTF2H1
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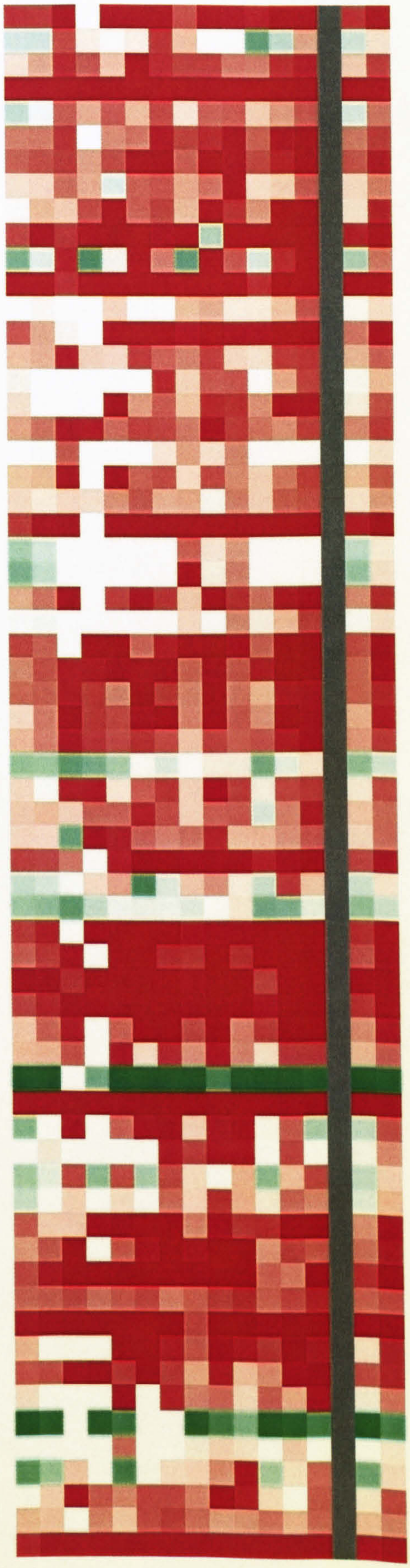
PCQAP
HDAC2
ABP1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VIM
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ITGB7
NR3C1
NEU1
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AA284856
INSR
AA281945
NME1
ANXA7
HSPG2
C1R
RPA1
MME
HNT
ADAR
KCNK1
POLR2A
ST14
BAK1
RAD23B
MYLK
DSIPI
RAD9
SH3GL2
FUBP1
UBTF
STAT1
H3F3B
MGC45562
SMARCA2
CHERP
SRP68
HLA-C
ADARB1
MINK
SDHA
PDCD4
CLN2
YWHAH
AA775803
TLR2
PGK1
PNLIPRP1
ARCN1
FLRT3
ASAH1
KLF1
DHPS
SPAG8
TBCE
G22P1

3.3 Table of apoptotic genes with significantly increased polysome association in B-CLL cells when compared to tonsil CD19+ B cells.

Cluster Number	Gene Name	Gene Description
Hs.80658	UCP2	uncoupling protein 2 (mitochondrial, proton carrier)
Hs.89414	CXCR4	chemokine (C-X-C motif) receptor 4
Hs.315463	IL24	interleukin 24
Hs.11171	APG5L	APG5 autophagy 5-like (S. cerevisiae)
Hs.315463	IL24	interleukin 24
Hs.410488	R39356	EST, Weakly similar to hypothetical protein FLJ20489 [Homo sapiens] [H.sapiens]
Hs.12409	SST	somatostatin
Hs.79241	BCL2	B-cell CLL/lymphoma 2
Hs.79391	HD	huntingtin (Huntington disease)
Hs.169449	PRKCA	protein kinase C, alpha
Hs.12272	BECN1	beclin 1 (coiled-coil, myosin-like BCL2 interacting protein)
Hs.93213	BAK1	BCL2-antagonist/killer 1
Hs.326248	PDCD4	programmed cell death 4 (neoplastic transformation inhibitor)

3.4 Table of cell cycle genes with significantly increased polysome association in B-CLL cells when compared to tonsil CD19+ B cells.

Cluster Number	Gene Name	Gene Description
Hs.95577	CDK4	cyclin-dependent kinase 4
Hs.183684	EIF4G2	eukaryotic translation initiation factor 4 gamma, 2
Hs.410488	R39356	EST, Weakly similar to hypothetical protein FLJ20489 [Homo sapiens] [H.sapiens]
Hs.155595	NEDD5	neural precursor cell expressed, developmentally down-regulated 5
Hs.74405	YWHAQ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide
Hs.79241	BCL2	B-cell CLL/lymphoma 2
Hs.75188	WEE1	WEE1 homolog (S. pombe)
Hs.252280	ARHGEF1	Rho guanine nucleotide exchange factor (GEF) 1
Hs.169449	PRKCA	protein kinase C, alpha
Hs.13512	ZW10	ZW10 homolog, centromere/kinetochore protein (Drosophila)
Hs.179565	MCM3	MCM3 minichromosome maintenance deficient 3 (S. cerevisiae)
Hs.118638	NME1	non-metastatic cells 1, protein (NM23A) expressed in
Hs.21486	STAT1	signal transducer and activator of transcription 1, 91kDa

3.5 Table of proliferation genes with significantly increased polysome association in B-CLL cells when compared to tonsil CD19+ B cells.

Cluster Number	Gene Name	Gene Description
Hs.11101	DLG3	discs, large (Drosophila) homolog 3 (neuroendocrine-dlg)
Hs.84	IL2RG	interleukin 2 receptor, gamma (severe combined immunodeficiency)
Hs.77054	BTG1	B-cell translocation gene 1, anti-proliferative
Hs.95577	CDK4	cyclin-dependent kinase 4
Hs.374466	WARS	tryptophanyl-tRNA synthetase
Hs.123078	TSHR	thyroid stimulating hormone receptor
Hs.12409	SST	somatostatin
Hs.79241	BCL2	B-cell CLL/lymphoma 2
Hs.75140	LRPAP1	low density lipoprotein-related protein-associated protein 1 (alpha-2-macroglobulin receptor-associated protein 1)
Hs.252280	ARHGEF1	Rho guanine nucleotide exchange factor (GEF) 1
Hs.169449	PRKCA	protein kinase C, alpha
Hs.75576	PLG	plasminogen
Hs.118638	NME1	non-metastatic cells 1, protein (NM23A) expressed in
Hs.431496	AA775803	EST, Moderately similar to I68897 probable thioredoxin peroxidase (EC 1.11.1.-) 1 - human [H.sapiens]
Hs.79064	DHPS	deoxyhypusine synthase

3.6 Table of receptor genes with significantly increased polysome association in B-CLL cells when compared to tonsil CD19+ B cells.

Cluster Number	Gene Name	Gene Description
Hs.89472	AGTR1	angiotensin II receptor, type 1
Hs.89414	CXCR4	chemokine (C-X-C motif) receptor 4
Hs.95327	CD3D	CD3D antigen, delta polypeptide (TiT3 complex)
Hs.84	IL2RG	interleukin 2 receptor, gamma (severe combined immunodeficiency)
Hs.856	IFNG	interferon, gamma
Hs.80887	LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog
Hs.1790	NR3C2	nuclear receptor subfamily 3, group C, member 2
Hs.277477	HLA-C	major histocompatibility complex, class I, C
Hs.376966	AA035450	Homo sapiens mRNA; cDNA DKFZp313N1434 (from clone DKFZp313N1434), mRNA sequence
Hs.123078	TSHR	thyroid stimulating hormone receptor
Hs.12409	SST	somatostatin
Hs.380986	MGC16723	hypothetical protein MGC16723
Hs.159553	CMKLR1	chemokine-like receptor 1
Hs.75140	LRPAP1	low density lipoprotein-related protein-associated protein 1 (alpha-2-macroglobulin receptor-associated protein 1)
Hs.748	FGFR1	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)
Hs.169449	PRKCA	protein kinase C, alpha
Hs.1741	ITGB7	integrin, beta 7
Hs.75772	NR3C1	nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
Hs.89695	INSR	insulin receptor
Hs.376459	AA281945	Homo sapiens cDNA FLJ36300 fis, clone THYMU2004410, mRNA sequence
Hs.21486	STAT1	signal transducer and activator of transcription 1, 91kDa
Hs.6335	MGC45562	hypothetical protein MGC45562
Hs.277477	HLA-C	major histocompatibility complex, class I, C
Hs.63668	TLR2	toll-like receptor 2
Hs.41296	FLRT3	fibronectin leucine rich transmembrane protein 3

3.7 Table of cell signalling genes with significantly increased polysome association in B-CLL cells when compared to tonsil CD19+ B cells.

Cluster Number	Gene Name	Gene Description
Hs.17165	RGS13	regulator of G-protein signalling 13
Hs.95327	CD3D	CD3D antigen, delta polypeptide (TiT3 complex)
Hs.84	IL2RG	interleukin 2 receptor, gamma (severe combined immunodeficiency)
Hs.35140	STK4	serine/threonine kinase 4
Hs.77202	PRKCB1	protein kinase C, beta 1
Hs.78877	ITPKB	inositol 1,4,5-trisphosphate 3-kinase B
Hs.856	IFNG	interferon, gamma
Hs.165843	CSNK2B	casein kinase 2, beta polypeptide
Hs.1790	NR3C2	nuclear receptor subfamily 3, group C, member 2
Hs.81008	FLNB	filamin B, beta (actin binding protein 278)
Hs.376966	AA035450	Homo sapiens mRNA; cDNA DKFZp313N1434 (from clone DKFZp313N1434), mRNA sequence
Hs.252280	ARHGEF1	Rho guanine nucleotide exchange factor (GEF) 1
Hs.155206	STK25	serine/threonine kinase 25 (STE20 homolog, yeast)
Hs.169449	PRKCA	protein kinase C, alpha
Hs.174044	DVL3	dishevelled, dsh homolog 3 (Drosophila)
Hs.83656	ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta
Hs.75772	NR3C1	nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
Hs.89695	INSR	insulin receptor
Hs.376459	AA281945	Homo sapiens cDNA FLJ36300 fis, clone THYMU2004410, mRNA sequence
Hs.75149	SH3GL2	SH3-domain GRB2-like 2
Hs.21486	STAT1	signal transducer and activator of transcription 1, 91kDa
Hs.6335	MGC45562	hypothetical protein MGC45562
Hs.41296	FLRT3	fibronectin leucine rich transmembrane protein 3

3.8 Table of translation associated genes with significantly increased polysome association in B-CLL cells when compared to tonsil CD19+ B cells.

Cluster Number	Gene Name	Gene Description
Hs.183684	EIF4G2	eukaryotic translation initiation factor 4 gamma, 2
Hs.297753	VIM	vimentin
Hs.337766	RPL18A	ribosomal protein L18a
Hs.273307	SRP68	signal recognition particle 68kDa

Appendix 4

4.1 Table of genes with significantly decreased polysome association when compared to tonsil CD19+ B cells.

Cluster Number	Gene Name	Gene Description
Hs.29352	TNFAIP6	tumor necrosis factor, alpha-induced protein 6
Hs.20894	NDST1	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1
Hs.75106	CLU	clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J)
Hs.42650	ZWINT	ZW10 interactor
Hs.95990	PKLR	pyruvate kinase, liver and RBC
Hs.380757	GYG2	glycogenin 2
Hs.2420	SOD3	superoxide dismutase 3, extracellular
Hs.12773	ACOX3	acyl-Coenzyme A oxidase 3, pristanoyl
Hs.29475	TYMS	thymidylate synthetase
Hs.381231	CASP8	caspase 8, apoptosis-related cysteine protease
Hs.331803	AI284071	ESTs, Highly similar to A32800 chaperonin GroEL precursor - human [H.sapiens]
Hs.417533	H17528	EST
Hs.114311	CDC45L	CDC45 cell division cycle 45-like (S. cerevisiae)
Hs.420151	SPINK1	serine protease inhibitor, Kazal type 1
Hs.351593	FGA	fibrinogen, A alpha polypeptide
Hs.4756	FEN1	flap structure-specific endonuclease 1
Hs.16269	BCL7B	B-cell CLL/lymphoma 7B
Hs.75318	TUBA1	tubulin, alpha 1 (testis specific)
Hs.79033	QPCT	glutaminyl-peptide cyclotransferase (glutaminyl cyclase)
Hs.406367	AA491213	Unknown (protein for IMAGE:4822098) [Homo sapiens], mRNA sequence
Hs.180655	STK12	serine/threonine kinase 12
Hs.251653	TUBB2	tubulin, beta, 2
Hs.33021	NOVA2	neuro-oncological ventral antigen 2
Hs.166684	STK3	serine/threonine kinase 3 (STE20 homolog, yeast)
Hs.42826	RBM4	RNA binding motif protein 4
Hs.77541	ARF5	ADP-ribosylation factor 5
Hs.111024	SLC25A1	solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1
Hs.89571	RAD52	RAD52 homolog (S. cerevisiae)
Hs.75074	MAPKAPK2	mitogen-activated protein kinase-activated protein kinase 2
Hs.80741	PCCA	propionyl Coenzyme A carboxylase, alpha polypeptide
Hs.14623	IFI30	interferon, gamma-inducible protein 30
Hs.30954	PMVK	phosphomevalonate kinase
Hs.46423	H4FG	H4 histone family, member G
Hs.377973	LMNA	lamin A/C
Hs.63525	PCBP2	poly(rC) binding protein 2
Hs.75893	ANK3	ankyrin 3, node of Ranvier (ankyrin G)
Hs.305890	BCL2L1	BCL2-like 1
Hs.288544	RPL35A	ribosomal protein L35a
Hs.93837	PITPNM	phosphatidylinositol transfer protein, membrane-associated
Hs.433506	AA188155	Homo sapiens, clone IMAGE:3457786, mRNA, mRNA sequence
Hs.118787	TGFBI	transforming growth factor, beta-induced, 68kDa
Hs.1376	HSD11B2	hydroxysteroid (11-beta) dehydrogenase 2
Hs.37045	PTH	parathyroid hormone
Hs.391848	H22826	ESTs, Highly similar to LIM domain only 7 isoform b [Homo sapiens] [H.sapiens]

Hs.76325	IGJ	immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides
Hs.180383	DUSP6	dual specificity phosphatase 6
Hs.78452	SLC20A1	solute carrier family 20 (phosphate transporter), member 1
Hs.75748	PSMB1	proteasome (prosome, macropain) subunit, beta type, 1
Hs.251850	PSG5	pregnancy specific beta-1-glycoprotein 5
Hs.179665	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
Hs.326709	AA867984	Similar to phospholipase A2, group IB (pancreas) [Homo sapiens], mRNA sequence
Hs.80343	MMP15	matrix metalloproteinase 15 (membrane-inserted)
Hs.271980	MAPK6	mitogen-activated protein kinase 6
Hs.2794	DC12	DC12 protein
Hs.99855	FPRL1	formyl peptide receptor-like 1
Hs.172458	IDS	iduronate 2-sulfatase (Hunter syndrome)
Hs.179825	RANBP2L1	RAN binding protein 2-like 1
Hs.102598	MADCAM1	mucosal vascular addressin cell adhesion molecule 1
Hs.119007	RAB4A	RAB4A, member RAS oncogene family
Hs.234734	LYZ	lysozyme (renal amyloidosis)
Hs.2934	RRM1	ribonucleotide reductase M1 polypeptide
Hs.279910	ATOX1	ATX1 antioxidant protein 1 homolog (yeast)
Hs.173724	CKB	creatine kinase, brain
Hs.246857	MAPK9	mitogen-activated protein kinase 9
Hs.40300	CAPN3	calpain 3, (p94)
Hs.92137	R62862	ESTs, Highly similar to MYCL_HUMAN L-myc-1 proto-oncogene protein [H.sapiens]
Hs.233952	PSMA7	proteasome (prosome, macropain) subunit, alpha type, 7
Hs.423633	N95165	EST
Hs.166468	PDCD5	programmed cell death 5
Hs.169294	TCF7	transcription factor 7 (T-cell specific, HMG-box)
Hs.121017	H2AFA	H2A histone family, member A
Hs.1513	IFNAR1	interferon (alpha, beta and omega) receptor 1
Hs.70983	PARG1	PTPL1-associated RhoGAP 1
Hs.79348	RGS7	regulator of G-protein signalling 7
Hs.30888	COX7A2L	cytochrome c oxidase subunit VIIa polypeptide 2 like
Hs.93210	C8A	complement component 8, alpha polypeptide
Hs.278275	TPSG1	tryptase gamma 1
Hs.1519	PRKAR1B	protein kinase, cAMP-dependent, regulatory, type I, beta
Hs.5462	SLC4A4	solute carrier family 4, sodium bicarbonate cotransporter, member 4
Hs.88974	CYBB	cytochrome b-245, beta polypeptide (chronic granulomatous disease)
Hs.82483	MADH2	MAD, mothers against decapentaplegic homolog 2 (Drosophila)
Hs.6453	ITPK1	inositol 1,3,4-triphosphate 5/6 kinase
Hs.73851	ATP5J	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit F6
Hs.76297	GPRK6	G protein-coupled receptor kinase 6
Hs.433506	AA188155	Homo sapiens, clone IMAGE:3457786, mRNA, mRNA sequence
Hs.82927	AMPD2	adenosine monophosphate deaminase 2 (isoform L)
Hs.477	HSD17B3	hydroxysteroid (17-beta) dehydrogenase 3
Hs.19699	CGTHBA	Conserved gene telomeric to alpha globin cluster
Hs.406521	AA862813	ESTs, Highly similar to 1501259A cytochrome c oxidase VIII [Homo sapiens] [H.sapiens]
Hs.104	HGFAC	HGF activator
Hs.26776	NTRK3	neurotrophic tyrosine kinase, receptor, type 3
Hs.389933	RPS4X	ribosomal protein S4, X-linked
Hs.374491	PA2G4	proliferation-associated 2G4, 38kDa
Hs.356739	PPP6C	protein phosphatase 6, catalytic subunit
Hs.58169	HEC	highly expressed in cancer, rich in leucine heptad repeats

Hs.241572	GOLGA5	golgi autoantigen, golgin subfamily a, 5
Hs.63525	PCBP2	poly(rC) binding protein 2
Hs.83765	DHFR	dihydrofolate reductase
Hs.35120	RFC4	replication factor C (activator 1) 4, 37kDa
Hs.6755	RPIP8	RaP2 interacting protein 8
Hs.422953	N63107	EST
Hs.372755	KDELR2	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2
Hs.12210	TEM6	tumor endothelial marker 6
Hs.89455	OPRK1	opioid receptor, kappa 1
Hs.367900	PDCD2	programmed cell death 2
Hs.173824	TDG	thymine-DNA glycosylase
Hs.2233	CSF3	colony stimulating factor 3 (granulocyte)
Hs.432833	UBE2I	ubiquitin-conjugating enzyme E2I (UBC9 homolog, yeast)
Hs.153591	NOT56L	Not56 (D. melanogaster)-like protein
Hs.397980	SLC25A6	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6
Hs.275182	PIP5K1C	phosphatidylinositol-4-phosphate 5-kinase, type I, gamma
Hs.226795	GSTP1	glutathione S-transferase pi
Hs.77348	HPGD	hydroxyprostaglandin dehydrogenase 15-(NAD)
Hs.76244	SRM	spermidine synthase
Hs.74647	TRA@	T cell receptor alpha locus
Hs.194772	OMG	oligodendrocyte myelin glycoprotein
Hs.155894	PTPN1	protein tyrosine phosphatase, non-receptor type 1
Hs.74592	SATB1	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)
Hs.239818	PIK3CB	phosphoinositide-3-kinase, catalytic, beta polypeptide
Hs.74369	ITGA7	integrin, alpha 7
Hs.198365	BPGM	2,3-bisphosphoglycerate mutase
Hs.82071	CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2
Hs.250769	ALPL	alkaline phosphatase, liver/bone/kidney
Hs.423103	HPIP	HCF-1 beta-propeller interacting protein
Hs.278443	FCGR2B	Fc fragment of IgG, low affinity IIb, receptor for (CD32)
Hs.348935	IGLL1	immunoglobulin lambda-like polypeptide 1
Hs.433603	AA425973	ESTs, Moderately similar to KI67_HUMAN Antigen KI-67 [H.sapiens]
Hs.40866	KCNQ3	potassium voltage-gated channel, KQT-like subfamily, member 3
Hs.33084	SLC2A5	solute carrier family 2 (facilitated glucose/fructose transporter), member 5
Hs.169206	VTI1B	vesicle transport through interaction with t-SNAREs homolog 1B (yeast)
Hs.79410	SLC4A2	solute carrier family 4, anion exchanger, member 2 (erythrocyte membrane protein band 3-like 1)
Hs.2726	HMGA2	high mobility group AT-hook 2
Hs.7647	MAZ	MYC-associated zinc finger protein (purine-binding transcription factor)
Hs.287115	ECE1	endothelin converting enzyme 1
Hs.321197	INADL	PDZ domain protein (Drosophila inaD-like)
Hs.34114	ATP1A2	ATPase, Na+/K+ transporting, alpha 2 (+) polypeptide
Hs.2994	PCTK3	PCTAIRE protein kinase 3
Hs.367762	KRT6A	keratin 6A
Hs.143482	PPID	peptidylprolyl isomerase D (cyclophilin D)
Hs.348935	IGLL1	immunoglobulin lambda-like polypeptide 1
Hs.89571	RAD52	RAD52 homolog (S. cerevisiae)
Hs.416948	H12312	ESTs
Hs.75823	AF1Q	ALL1-fused gene from chromosome 1q
Hs.173554	UQCRC2	ubiquinol-cytochrome c reductase core protein II

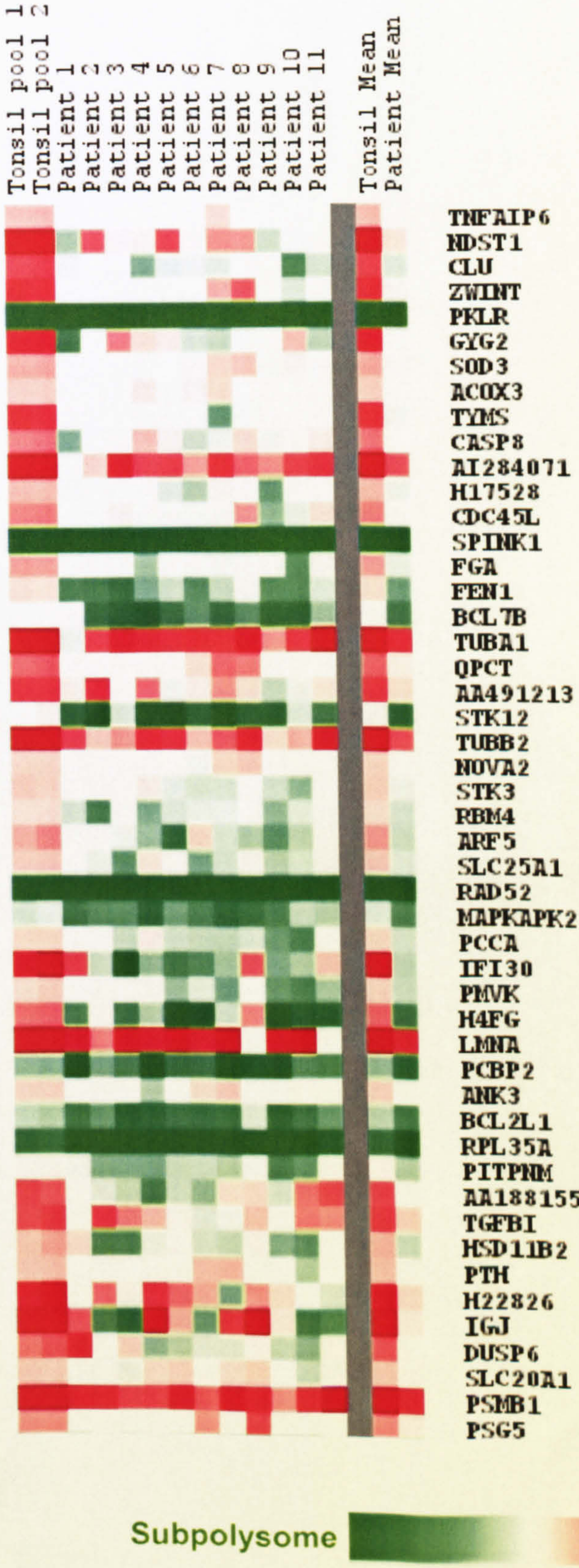
Hs.108966	PIP5K2A	phosphatidylinositol-4-phosphate 5-kinase, type II, alpha
Hs.183153	ARF4L	ADP-ribosylation factor 4-like
Hs.265829	ITGA3	integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)
Hs.418740	H18633	Homo sapiens cDNA FLJ39398 fis, clone PLACE6010704, mRNA sequence
Hs.367689	TRIO	triple functional domain (PTPRF interacting)
Hs.21	ELA2A	elastase 2A
Hs.73165	IL12RB2	interleukin 12 receptor, beta 2
Hs.82327	GSS	glutathione synthetase
Hs.2352	ADCY2	adenylate cyclase 2 (brain)
Hs.119014	ZNF175	zinc finger protein 175
Hs.77367	CXCL9	chemokine (C-X-C motif) ligand 9
Hs.433337	C20orf16	chromosome 20 open reading frame 16
Hs.84244	KCNB1	potassium voltage-gated channel, Shab-related subfamily, member 1
Hs.406455	PSAP	prosaposin (variant Gaucher disease and variant metachromatic leukodystrophy)
Hs.103502	GPT	glutamic-pyruvate transaminase (alanine aminotransferase)
Hs.79375	HLCS	holocarboxylase synthetase (biotin-[proprionyl-Coenzyme A-carboxylase (ATP-hydrolysing)] ligase)
Hs.35120	RFC4	replication factor C (activator 1) 4, 37kDa
Hs.271986	ITGA2	integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
Hs.54941	PHKA2	phosphorylase kinase, alpha 2 (liver)
Hs.110736	SLC12A2	solute carrier family 12 (sodium/potassium/chloride transporters), member 2
Hs.183123	NR5A2	nuclear receptor subfamily 5, group A, member 2
Hs.3631	IGBP1	immunoglobulin (CD79A) binding protein 1
Hs.356442	HDAC3	histone deacetylase 3
Hs.250712	CACNB3	calcium channel, voltage-dependent, beta 3 subunit
Hs.35101	PRRG2	proline-rich Gla (G-carboxyglutamic acid) polypeptide 2
Hs.78853	UNG	uracil-DNA glycosylase
Hs.153640	CNK	cytokine-inducible kinase
Hs.737	ETR101	immediate early protein
Hs.2943	SRP19	signal recognition particle 19kDa
Hs.350077	RPL21	ribosomal protein L21
Hs.285176	SLC33A1	solute carrier family 33 (acetyl-CoA transporter), member 1
Hs.283007	PLTP	phospholipid transfer protein
Hs.79110	NCL	nucleolin
Hs.83383	PRDX4	peroxiredoxin 4
Hs.34012	BRCA2	breast cancer 2, early onset
Hs.37040	PDGFA	platelet-derived growth factor alpha polypeptide
Hs.173162	NOC4	neighbor of COX4
Hs.172673	AHCY	S-adenosylhomocysteine hydrolase
Hs.81728	UNC119	unc-119 homolog (C. elegans)
Hs.432007	AA873577	EST, Moderately similar to ATPO_HUMAN ATP synthase oligomycin sensitivity conferral protein, mitochondrial precursor (OSCP) [H.sapiens]
Hs.87247	HRK	harakiri, BCL2 interacting protein (contains only BH3 domain)
Hs.211956	ASE-1	CD3-epsilon-associated protein; antisense to ERCC-1
Hs.249200	FGF19	fibroblast growth factor 19
Hs.73454	TNNT3	troponin T3, skeletal, fast
Hs.82283	MTR	5-methyltetrahydrofolate-homocysteine methyltransferase
Hs.127826	EPOR	erythropoietin receptor
Hs.64794	ZNF183	zinc finger protein 183 (RING finger, C3HC4 type)
Hs.404077	MGP	matrix Gla protein
Hs.110849	ESRRA	estrogen-related receptor alpha
Hs.364345	NNMT	nicotinamide N-methyltransferase

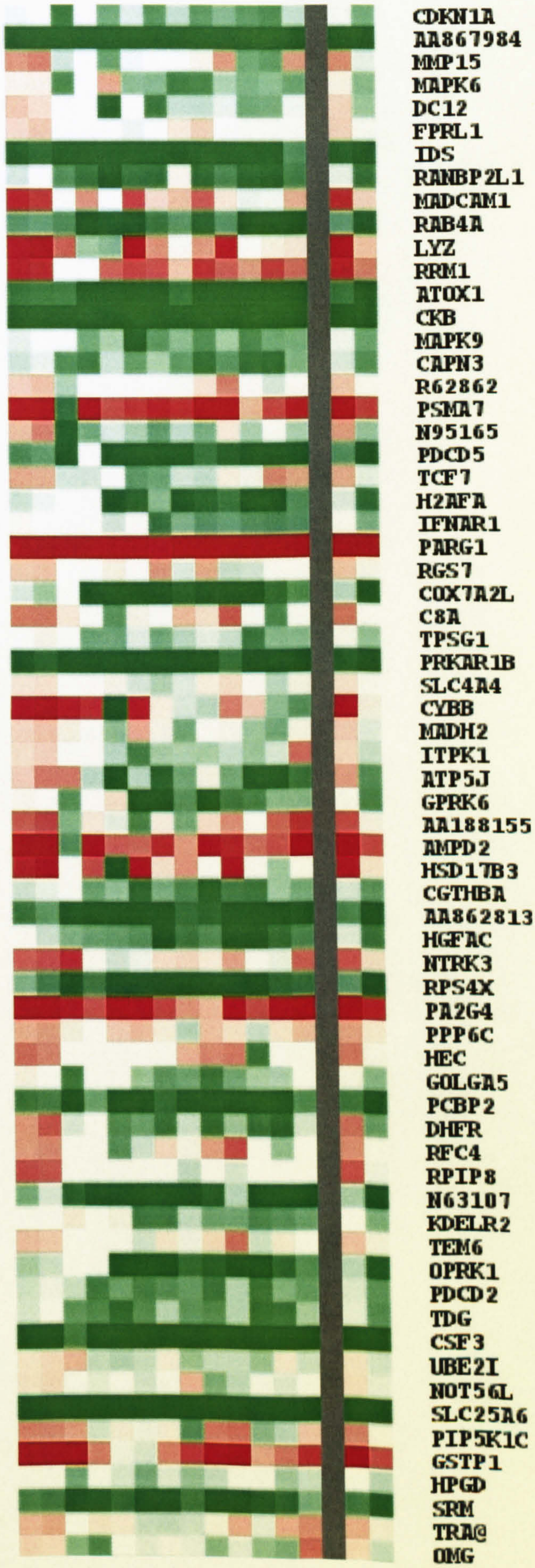
Hs.82401	CD69	CD69 antigen (p60, early T-cell activation antigen)
Hs.3281	NPTX2	neuronal pentraxin II
Hs.8372	UQCR	ubiquinol-cytochrome c reductase (6.4kD) subunit
Hs.143102	AOC2	amine oxidase, copper containing 2 (retina-specific)
Hs.55481	ZNF165	zinc finger protein 165
Hs.108809	CCT7	chaperonin containing TCP1, subunit 7 (eta)
Hs.115474	RFC3	replication factor C (activator 1) 3, 38kDa
Hs.377910	AA488588	Homo sapiens cDNA FLJ25478 fis, clone CBL03360, mRNA sequence
Hs.300697	IGHG3	immunoglobulin heavy constant gamma 3 (G3m marker)
Hs.171834	PCTK1	PCTAIRE protein kinase 1
Hs.74519	PRIM2A	primase, polypeptide 2A, 58kDa
Hs.86386	MCL1	myeloid cell leukemia sequence 1 (BCL2-related)
Hs.151777	EIF2S1	eukaryotic translation initiation factor 2, subunit 1 alpha, 35kDa
Hs.75969	PROL2	proline rich 2
Hs.1063	SNRPC	small nuclear ribonucleoprotein polypeptide C
Hs.79015	MOX2	antigen identified by monoclonal antibody MRC OX-2
Hs.17518	cig5	vipirin
Hs.80917	AP3S1	adaptor-related protein complex 3, sigma 1 subunit
Hs.388585	AA436278	ESTs, Weakly similar to hypothetical protein FLJ20489 [Homo sapiens] [H.sapiens]
Hs.347508	BG185544	Homo sapiens cDNA: FLJ23482 fis, clone KAIA03142, mRNA sequence
Hs.390278	PSG3	pregnancy specific beta-1-glycoprotein 3
Hs.389107	ATP6V0C	ATPase, H+ transporting, lysosomal 16kDa, V0 subunit c
Hs.49105	FAP48	FKBP-associated protein
Hs.2664	FMO4	flavin containing monooxygenase 4
Hs.155482	HAGH	hydroxyacyl glutathione hydrolase
Hs.102950	IGLJ3	immunoglobulin lambda joining 3
Hs.433750	EIF4G1	eukaryotic translation initiation factor 4 gamma, 1
Hs.3764	GUK1	guanylate kinase 1
Hs.2706	GPX4	glutathione peroxidase 4 (phospholipid hydroperoxidase)
Hs.421349	CDKN2A	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)
Hs.278611	GALNT3	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (GalNAc-T3)
Hs.155478	CCNT2	cyclin T2
Hs.1770	LIG1	ligase I, DNA, ATP-dependent
Hs.82793	PSMB3	proteasome (prosome, macropain) subunit, beta type, 3
Hs.432483	RQCD1	RCD1 required for cell differentiation1 homolog (S. pombe)
Hs.410626	AI363781	EST
Hs.355009	T65407	ESTs
Hs.279609	MTCH2	mitochondrial carrier homolog 2
Hs.338207	FRAP1	FK506 binding protein 12-rapamycin associated protein 1
Hs.27744	RAB3A	RAB3A, member RAS oncogene family
Hs.1042	SSA1	Sjogren syndrome antigen A1 (52kDa, ribonucleoprotein autoantigen SS-A/Ro)
Hs.90370	ARPC1A	actin related protein 2/3 complex, subunit 1A, 41kDa
Hs.429366	TXNL	thioredoxin-like, 32kDa
Hs.397609	RPS16	ribosomal protein S16
Hs.74362	CLPP	ClpP caseinolytic protease, ATP-dependent, proteolytic subunit homolog (E. coli)
Hs.109752	RCL	putative c-Myc-responsive
Hs.75087	FASTK	FAST kinase
Hs.129844	TNFRSF10D	tumor necrosis factor receptor superfamily, member 10d, decoy with truncated death domain
Hs.155324	MMP11	matrix metalloproteinase 11 (stromelysin 3)
Hs.79933	CCNI	cyclin I

Hs.119324	KNSL4	kinesin-like 4
Hs.172772	TCEB2	transcription elongation factor B (SIII), polypeptide 2 (18kDa, elongin B)
Hs.160786	ASS	argininosuccinate synthetase
Hs.151301	CADPS	Ca2+-dependent activator protein for secretion
Hs.169900	PABPC4	poly(A) binding protein, cytoplasmic 4 (inducible form)
Hs.112058	SIVA	CD27-binding (Siva) protein
Hs.78921	AKAP1	A kinase (PRKA) anchor protein 1
Hs.198282	PLSCR1	phospholipid scramblase 1
Hs.155553	HNK-1ST	HNK-1 sulfotransferase
Hs.181390	CSNK1G2	casein kinase 1, gamma 2
Hs.79078	MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)
Hs.2913	EPHB3	EphB3
Hs.83173	CCND3	cyclin D3
Hs.122579	ECT2	epithelial cell transforming sequence 2 oncogene
Hs.8724	STK38	serine/threonine kinase 38
Hs.150917	CTNNA2	catenin (cadherin-associated protein), alpha 2
Hs.173993	RBM6	RNA binding motif protein 6
Hs.75251	PIAS1	protein inhibitor of activated STAT, 1
Data not found	H69630	
Hs.77329	PTDSS1	phosphatidylserine synthase 1
Hs.83848	TPI1	triosephosphate isomerase 1
Hs.927	MYBPH	myosin binding protein H
Hs.7768	FIBP	fibroblast growth factor (acidic) intracellular binding protein
Hs.76536	TBL1X	transducin (beta)-like 1X-linked
Hs.80828	KRT1	keratin 1 (epidermolytic hyperkeratosis)
Hs.25537	CTF1	cardiotrophin 1
Hs.184014	RPL31	ribosomal protein L31
Hs.68257	GTF2F1	general transcription factor IIF, polypeptide 1, 74kDa
Hs.211773	CHES1	checkpoint suppressor 1
Hs.75655	P4HB	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), beta polypeptide (protein disulfide isomerase; thyroid hormone binding protein p55)
Hs.44499	PNN	pinin, desmosome associated protein
Hs.82963	GNRH1	gonadotropin-releasing hormone 1 (leutinizing-releasing hormone)
Hs.29222	ZNF76	zinc finger protein 76 (expressed in testis)
Hs.104119	RAGE	renal tumor antigen
Hs.79322	QARS	glutaminyl-tRNA synthetase
Hs.433482	NDUFB7	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7, 18kDa
Hs.334895	RPL10A	ribosomal protein L10a
Hs.177559	IFNGR2	interferon gamma receptor 2 (interferon gamma transducer 1)
Hs.113207	GPR30	G protein-coupled receptor 30
Hs.406631	CDC27	cell division cycle 27
Hs.355934	CARP	cardiac ankyrin repeat protein
Hs.75212	ODC1	ornithine decarboxylase 1
Hs.170917	PTGER3	prostaglandin E receptor 3 (subtype EP3)
Hs.406171	SMA5	SMA5
Hs.73737	SFRS1	splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)
Hs.28914	APRT	adenine phosphoribosyltransferase
Hs.2706	GPX4	glutathione peroxidase 4 (phospholipid hydroperoxidase)
Hs.300280	AMY2A	amylase, alpha 2A; pancreatic
Hs.96448	ZNF193	zinc finger protein 193
Hs.343575	ABI-2	abl-interactor 2
Hs.41587	RAD50	RAD50 homolog (S. cerevisiae)
Hs.82280	RGS10	regulator of G-protein signalling 10
Hs.24976	ART3	ADP-ribosyltransferase 3

Hs.73965	SFRS2	splicing factor, arginine/serine-rich 2
Hs.101382	TNFAIP2	tumor necrosis factor, alpha-induced protein 2
Hs.114408	TLR5	toll-like receptor 5
Hs.356176	ACAA2	acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)
Hs.81071	ECM1	extracellular matrix protein 1
Hs.110903	CLDN5	claudin 5 (transmembrane protein deleted in velocardiofacial syndrome)
Hs.79357	PSMC6	proteasome (prosome, macropain) 26S subunit, ATPase, 6
Hs.122511	CETN1	centrin, EF-hand protein, 1
Hs.79946	CYP19	cytochrome P450, subfamily XIX (aromatization of androgens)
Hs.193974	GSR	glutathione reductase
Hs.519	WWOX	WW domain containing oxidoreductase
Hs.90559	T50397	ESTs
Hs.22826	TMOD3	tropomodulin 3 (ubiquitous)
Hs.10237	ABCG1	ATP-binding cassette, sub-family G (WHITE), member 1
Hs.31472	TNFAIP6	tumor necrosis factor, alpha-induced protein 6

4.2 Genes with significantly decreased polysome association in B-CLL cells when compared to tonsil CD19+ B cells. A colour scale has been used to represent the ratio of subpolysome to polysome message for each tonsil and B-CLL patient sample. Where the square is red the message is more polysome associated and where green more subpolysome associated.

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MAPK6
DC12
FPRL1
IDS
RANBP2L1
MADCAM1
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LYZ
RRM1
ATOX1
CKB
MAPK9
CAPN3
R62862
PSMA7
N95165
PDCD5
TCF7
H2AFA
IFNAR1
PARG1
RGS7
COX7A2L
C8A
TPSG1
PRKAR1B
SLC4A4
CYBB
MADH2
ITPK1
ATP5J
GPRK6
AA188155
AMPD2
HSD17B3
CGTHBA
AA862813
HGFAC
NTRK3
RPS4X
PA2G4
PPP6C
HEC
GOLGA5
PCBP2
DHFR
RFC4
RPIP8
N63107
KDELR2
TEM6
OPRK1
PDCD2
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UBE2I
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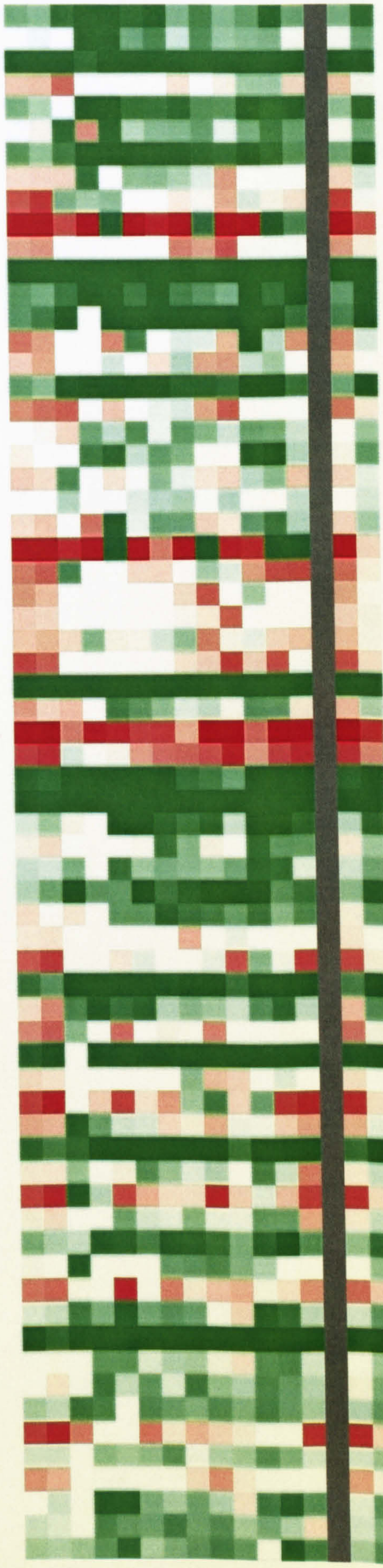
HPGD
SRM
TRA@
OMG



PTPN1
SATB1
PIK3CB
ITGA7
BPGM
CITED2
ALPL
HPIP
FCGR2B
IGLL1
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KCNQ3
SLC2A5
VTI1B
SLC4A2
HMGA2
MAZ
ECE1
INADL
ATP1A2
PCTK3
KRT6A
PPID
IGLL1
RAD52
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AF1Q
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PIP5K2A
ARF4L
ITGA3
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TRIO
ELA2A
IL12RB2
GSS
ADCY2
ZNF175
CXCL9
C20orf16
KCNB1
PSAP
GPT
HLCS
RFC4
ITGA2
PHKA2
SLC12A2
NR5A2
IGBP1
HDAC3
CACNB3
PRRG2
UNG
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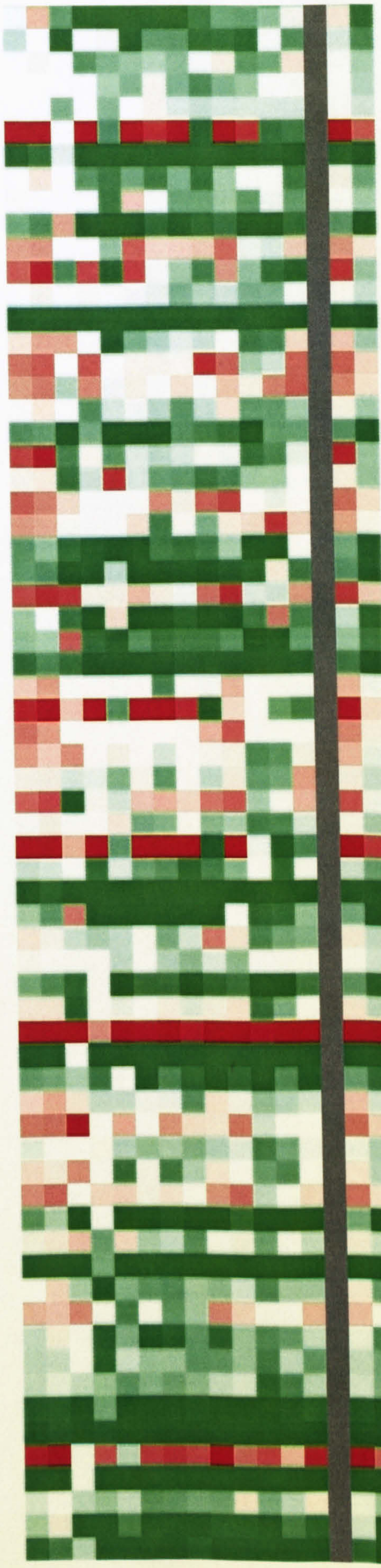

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FGF19
TNNT3
MTR
EPOR
ZNF183
MGP
ESRRA
NNMT
CD69
NPTX2
UQCR
AOC2
ZNF165
CCT7
RFC3
AA488588
IGHG3
PCTK1
PRIM2A
MCL1
EIF2S1
PROL2
SNRPC
MOX2
cig5
AP3S1
AA436278
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PSG3
ATP6V0C
FAP48
FMO4
HAGH
IGLJ3
EIF4G1
GUK1
GPX4
CDKN2A
GALNT3
CCNT2
LIG1
PSMB3
RQCD1
AI363781
T65407
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FRAP1
RAB3A
SSA1
ARPC1A
TXNL
RPS16
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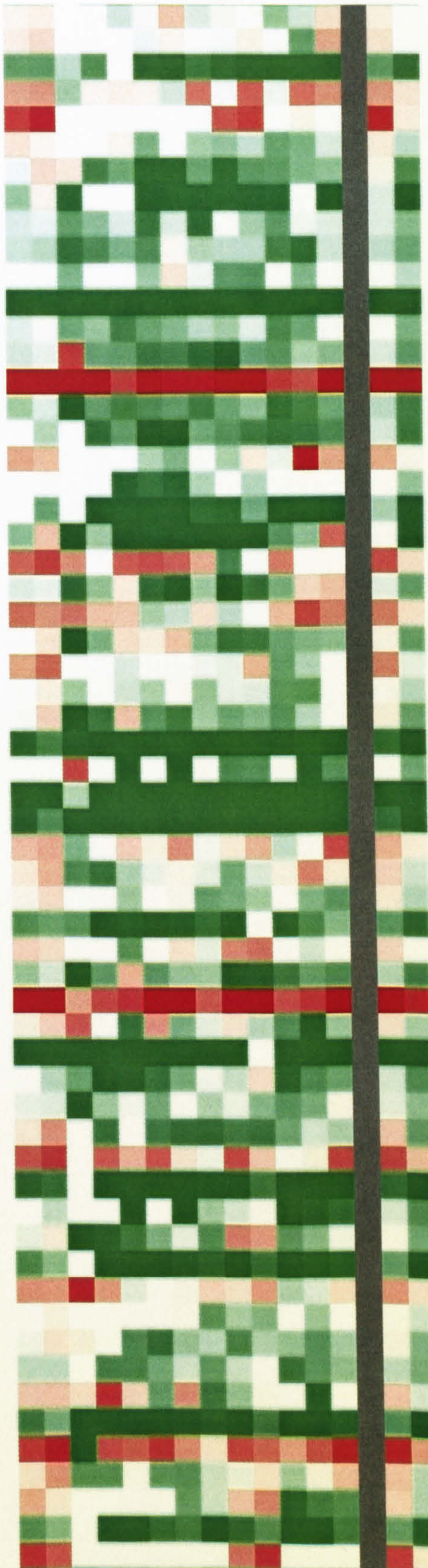
SIVA



AKAP1
PLSCR1
HNK-1ST
CSNK1G2
MAD2L1
EPHB3
CCND3
ECT2
STK38
CTNNA2
RBM6
PIAS1
H69630
PTDSS1
TPI1
MYBPH
FIBP
TBL1X
KRT1
CTF1
RPL31
GTF2F1
CHES1
P4HB
PNN
GNRH1
ZNF76
RAGE
QARS
NDUFB7
RPL10A
IFNGR2
GPR30
CDC27
CARP
ODC1
PTGER3
SMA5
SFRS1
APRT
GPX4
AMY2A
ZNF193
ABI-2
RAD50
RGS10
ART3
SFRS2
TNFAIP2
TLR5
ACAA2
ECM1
CLDN5
PSMC6
CETN1
CYP19
GSR
WWOX
T50397
TMOD3
ABCG1

4.3 Table of apoptotic genes with significantly decreased polysome association when compared to tonsil CD19+ B cells.

Cluster Number	Gene Name	Gene Description
Hs.75106	CLU	clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J)
Hs.166684	STK3	serine/threonine kinase 3 (STE20 homolog, yeast)
Hs.305890	BCL2L1	BCL2-like 1
Hs.37045	PTH	parathyroid hormone
Hs.180383	DUSP6	dual specificity phosphatase 6
Hs.179665	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
Hs.166468	PDCD5	programmed cell death 5
Hs.367900	PDCD2	programmed cell death 2
Hs.356442	HDAC3	histone deacetylase 3
Hs.34012	BRCA2	breast cancer 2, early onset
Hs.87247	HRK	harakiri, BCL2 interacting protein (contains only BH3 domain)
Hs.429366	TXNL	thioredoxin-like, 32kDa
Hs.75087	FASTK	FAST kinase
Hs.129844	TNFRSF10D	tumor necrosis factor receptor superfamily, member 10d, decoy with truncated death domain
Hs.112058	SIVA	CD27-binding (Siva) protein

4.4 Table of cell cycle genes with significantly decreased polysome association when compared to tonsil CD19+ B cells.

Cluster Number	Gene Name	Gene Description
Hs.114311	CDC45L	CDC45 cell division cycle 45-like (S. cerevisiae)
Hs.180655	STK12	serine/threonine kinase 12
Hs.180383	DUSP6	dual specificity phosphatase 6
Hs.179665	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
Hs.271980	MAPK6	mitogen-activated protein kinase 6
Hs.389933	RPS4X	ribosomal protein S4, X-linked
Hs.374491	PA2G4	proliferation-associated 2G4, 38kDa
Hs.356739	PPP6C	protein phosphatase 6, catalytic subunit
Hs.239818	PIK3CB	phosphoinositide-3-kinase, catalytic, beta polypeptide
Hs.433603	AA425973	ESTs, Moderately similar to KI67_HUMAN Antigen KI-67 [H.sapiens]
Hs.356442	HDAC3	histone deacetylase 3
Hs.153640	CNK	cytokine-inducible kinase
Hs.34012	BRCA2	breast cancer 2, early onset
Hs.37040	PDGFA	platelet-derived growth factor alpha polypeptide
Hs.108809	CCT7	chaperonin containing TCP1, subunit 7 (eta)
Hs.171834	PCTK1	PCTAIRE protein kinase 1
Hs.421349	CDKN2A	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)
Hs.155478	CCNT2	cyclin T2
Hs.338207	FRAP1	FK506 binding protein 12-rapamycin associated protein 1
Hs.79078	MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)
Hs.83173	CCND3	cyclin D3
Hs.211773	CHES1	checkpoint suppressor 1

4.5 Table of proliferation genes with significantly decreased polysome association when compared to tonsil CD19+ B cells.

Cluster Number	Gene Name	Gene Description
Hs.351593	FGA	fibrinogen, A alpha polypeptide
Hs.118787	TGFBI	transforming growth factor, beta-induced, 68kDa
Hs.179665	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
Hs.389933	RPS4X	ribosomal protein S4, X-linked
Hs.374491	PA2G4	proliferation-associated 2G4, 38kDa
Hs.2233	CSF3	colony stimulating factor 3 (granulocyte)
Hs.73165	IL12RB2	interleukin 12 receptor, beta 2
Hs.37040	PDGFA	platelet-derived growth factor alpha polypeptide
Hs.421349	CDKN2A	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)
Hs.109752	RCL	putative c-Myc-responsive
Hs.82963	GNRH1	gonadotropin-releasing hormone 1 (leutinizing-releasing hormone)
Hs.406631	CDC27	cell division cycle 27

4.6 Table of receptor genes with significantly decreased polysome association when compared to tonsil CD19+ B cells.

Cluster Number	Gene Name	Gene Description
Hs.29352	TNFAIP6	tumor necrosis factor, alpha-induced protein 6
Hs.37045	PTH	parathyroid hormone
Hs.78452	SLC20A1	solute carrier family 20 (phosphate transporter), member 1
Hs.99855	FPRL1	formyl peptide receptor-like 1
Hs.102598	MADCAM1	mucosal vascular addressin cell adhesion molecule 1
Hs.1513	IFNAR1	interferon (alpha, beta and omega) receptor 1
Hs.79348	RGS7	regulator of G-protein signalling 7
Hs.76297	GPRK6	G protein-coupled receptor kinase 6
Hs.26776	NTRK3	neurotrophic tyrosine kinase, receptor, type 3
Hs.372755	KDELR2	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2
Hs.89455	OPRK1	opioid receptor, kappa 1
Hs.2233	CSF3	colony stimulating factor 3 (granulocyte)
Hs.74647	TRA@	T cell receptor alpha locus
Hs.239818	PIK3CB	phosphoinositide-3-kinase, catalytic, beta polypeptide
Hs.74369	ITGA7	integrin, alpha 7
Hs.278443	FCGR2B	Fc fragment of IgG, low affinity IIb, receptor for (CD32)
Hs.169206	VTI1B	vesicle transport through interaction with t-SNAREs homolog 1B (yeast)
Hs.265829	ITGA3	integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)
Hs.367689	TRIO	triple functional domain (PTPRF interacting)
Hs.73165	IL12RB2	interleukin 12 receptor, beta 2
Hs.77367	CXCL9	chemokine (C-X-C motif) ligand 9
Hs.271986	ITGA2	integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
Hs.183123	NR5A2	nuclear receptor subfamily 5, group A, member 2
Hs.37040	PDGFA	platelet-derived growth factor alpha polypeptide
Hs.127826	EPOR	erythropoietin receptor
Hs.110849	ESRRA	estrogen-related receptor alpha
Hs.82401	CD69	CD69 antigen (p60, early T-cell activation antigen)
Hs.80917	AP3S1	adaptor-related protein complex 3, sigma 1 subunit
Hs.388585	AA436278	ESTs, Weakly similar to hypothetical protein FLJ20489 [Homo sapiens] [H.sapiens]
Hs.49105	FAP48	FKBP-associated protein
Hs.129844	TNFRSF10D	tumor necrosis factor receptor superfamily, member 10d, decoy with truncated death domain
Hs.122843	CASP8AP2	CASP8 associated protein 2
Hs.112058	SIVA	CD27-binding (Siva) protein
Hs.2913	EPHB3	EphB3
Hs.7768	FIBP	fibroblast growth factor (acidic) intracellular binding protein
Hs.177559	IFNGR2	interferon gamma receptor 2 (interferon gamma transducer 1)
Hs.113207	GPR30	G protein-coupled receptor 30
Hs.170917	PTGER3	prostaglandin E receptor 3 (subtype EP3)
Hs.114408	TLR5	toll-like receptor 5

4.7 Table of cell signalling genes with significantly decreased polysome association when compared to tonsil CD19+ B cells.

Cluster Number	Gene Name	Gene description
Hs.29352	TNFAIP6	tumor necrosis factor, alpha-induced protein 6
Hs.417533	H17528	EST
Hs.166684	STK3	serine/threonine kinase 3 (STE20 homolog, yeast)
Hs.77541	ARF5	ADP-ribosylation factor 5
Hs.271980	MAPK6	mitogen-activated protein kinase 6
Hs.102598	MADCAM1	mucosal vascular addressin cell adhesion molecule 1
Hs.119007	RAB4A	RAB4A, member RAS oncogene family
Hs.246857	MAPK9	mitogen-activated protein kinase 9
Hs.1513	IFNAR1	interferon (alpha, beta and omega) receptor 1
Hs.70983	PARG1	PTPL1-associated RhoGAP 1
Hs.1519	PRKAR1B	protein kinase, cAMP-dependent, regulatory, type I, beta
Hs.82483	MADH2	MAD, mothers against decapentaplegic homolog 2 (Drosophila)
Hs.6453	ITPK1	inositol 1,3,4-triphosphate 5/6 kinase
Hs.76297	GPRK6	G protein-coupled receptor kinase 6
Hs.6755	RPIP8	RaP2 interacting protein 8
Hs.422953	N63107	EST
Hs.2233	CSF3	colony stimulating factor 3 (granulocyte)
Hs.155894	PTPN1	protein tyrosine phosphatase, non-receptor type 1
Hs.239818	PIK3CB	phosphoinositide-3-kinase, catalytic, beta polypeptide
Hs.278443	FCGR2B	Fc fragment of IgG, low affinity IIb, receptor for (CD32)
Hs.183153	ARF4L	ADP-ribosylation factor 4-like
Hs.73165	IL12RB2	interleukin 12 receptor, beta 2
Hs.77367	CXCL9	chemokine (C-X-C motif) ligand 9
Hs.3631	IGBP1	immunoglobulin (CD79A) binding protein 1
Hs.37040	PDGFA	platelet-derived growth factor alpha polypeptide
Hs.127826	EPOR	erythropoietin receptor
Hs.82401	CD69	CD69 antigen (p60, early T-cell activation antigen)
Hs.27744	RAB3A	RAB3A, member RAS oncogene family
Hs.429366	TXNL	thioredoxin-like, 32kDa
Hs.75087	FASTK	FAST kinase
Hs.122843	CASP8AP2	CASP8 associated protein 2
Hs.181390	CSNK1G2	casein kinase 1, gamma 2
Hs.2913	EPHB3	EphB3
Hs.75251	PIAS1	protein inhibitor of activated STAT, 1
Hs.76536	TBL1X	transducin (beta)-like 1X-linked
Hs.82963	GNRH1	gonadotropin-releasing hormone 1 (leutinizing-releasing hormone)
Hs.177559	IFNGR2	interferon gamma receptor 2 (interferon gamma transducer 1)
Hs.355934	CARP	cardiac ankyrin repeat protein
Hs.82280	RGS10	regulator of G-protein signalling 10

4.8 Table of translation associated genes with significantly decreased polysome association when compared to tonsil CD19+ B cells.

Cluster Number	Gene Name	Gene Description
Hs.2943	SRP19	signal recognition particle 19kDa
Hs.151777	EIF2S1	eukaryotic translation initiation factor 2, subunit 1 alpha, 35kDa
Hs.433750	EIF4G1	eukaryotic translation initiation factor 4 gamma, 1
Hs.172772	TCEB2	transcription elongation factor B (SIII), polypeptide 2 (18kDa, elongin B)
Hs.288544	RPL35A	ribosomal protein L35a
Hs.389933	RPS4X	ribosomal protein S4, X-linked
Hs.350077	RPL21	ribosomal protein L21
Hs.397609	RPS16	ribosomal protein S16
Hs.184014	RPL31	ribosomal protein L31
Hs.334895	RPL10A	ribosomal protein L10a

Appendix 5

5.1 Table of genes with significantly increased total RNA levels in B-CLL cells when compared to tonsil CD19+ cells.

Cluster Number	Gene Name	Gene Description
Hs.79357	PSMC6	proteasome (prosome, macropain) 26S subunit, ATPase, 6
Hs.129882	IMPG1	interphotoreceptor matrix proteoglycan 1
Hs.348183	TNFRSF6B	tumor necrosis factor receptor superfamily, member 6b, decoy
Hs.256278	TNFRSF1B	tumor necrosis factor receptor superfamily, member 1B
Hs.77448	ALDH4A1	aldehyde dehydrogenase 4 family, member A1
Hs.258503	CYFIP2	cytoplasmic FMR1 interacting protein 2
Hs.4147	TRAM	translocating chain-associating membrane protein
Hs.150423	CDK9	cyclin-dependent kinase 9 (CDC2-related kinase)
Hs.74335	HSPCB	heat shock 90kDa protein 1, beta
Hs.809	HGF	hepatocyte growth factor (hepapoietin A; scatter factor)
Hs.74002	NCOA1	nuclear receptor coactivator 1
Hs.318501	TRIM22	tripartite motif-containing 22
Hs.76507	PIG7	LPS-induced TNF-alpha factor
Hs.74637	TEGT	testis enhanced gene transcript (BAX inhibitor 1)
Hs.11393	RAD51C	RAD51 homolog C (S. cerevisiae)
Hs.342874	TGFBR3	transforming growth factor, beta receptor III (betaglycan, 300kDa)
Hs.75438	QDPR	quinoid dihydropteridine reductase
Hs.28777	H2AFL	H2A histone family, member L
Hs.154210	EDG1	endothelial differentiation, sphingolipid G-protein-coupled receptor, 1
Hs.75149	SH3GL2	SH3-domain GRB2-like 2
Hs.74573	PLD3	likely ortholog of mouse phospholipase D3
Hs.373499	NSAP1	NS1-associated protein 1
Hs.115770	TNFSF11	tumor necrosis factor (ligand) superfamily, member 11
Hs.102456	SIP1	survival of motor neuron protein interacting protein 1
Hs.78281	RGS12	regulator of G-protein signalling 12
Hs.80409	GADD45A	growth arrest and DNA-damage-inducible, alpha
Hs.355866	ZNF148	zinc finger protein 148 (pHZ-52)
Hs.155956	NAT1	N-acetyltransferase 1 (arylamine N-acetyltransferase)
Hs.143288	MGC11271	hypothetical protein MGC11271
Hs.81875	GRB10	growth factor receptor-bound protein 10
Hs.80423	PBP	prostatic binding protein
Hs.251216	ESPN	espin
Hs.349650	RAF1	v-raf-1 murine leukemia viral oncogene homolog 1
Hs.146409	CDC42	cell division cycle 42 (GTP binding protein, 25kDa)
Hs.76686	GPX1	glutathione peroxidase 1
Hs.89472	AGTR1	angiotensin II receptor, type 1
Hs.79194	CREB1	cAMP responsive element binding protein 1
Hs.56937	ST14	suppression of tumorigenicity 14 (colon carcinoma, matriptase, epithin)
Hs.75969	PROL2	proline rich 2
Hs.41688	DUSP8	dual specificity phosphatase 8
Hs.326392	SOS1	son of sevenless homolog 1 (Drosophila)
Hs.376432	R16849	Homo sapiens cDNA FLJ36547 fis, clone TRACH2007577, mRNA sequence
Hs.172647	GOLGA1	golgi autoantigen, golgin subfamily a, 1
Hs.7019	SIPA1	signal-induced proliferation-associated gene 1
Hs.17518	cig5	vipirin

Hs.172690	DGKA	diacylglycerol kinase, alpha 80kDa
Hs.82527	SIAT8A	sialyltransferase 8A (alpha-N-acetylneuraminate: alpha-2,8-sialytransferase, GD3 synthase)
Hs.78146	PECAM1	platelet/endothelial cell adhesion molecule (CD31 antigen)
Hs.104633	AGRP	agouti related protein homolog (mouse)
Hs.184161	EXT1	exostoses (multiple) 1
Hs.408986	W49766	Homo sapiens cDNA FLJ33263 fis, clone ASTRO2006732, highly similar to WNT-5A PROTEIN PRECURSOR, mRNA sequence
Hs.103042	MAP1B	microtubule-associated protein 1B
Hs.174139	CLCN3	chloride channel 3
Hs.433875	T69304	ESTs, Moderately similar to P2G4_HUMAN Proliferation-associated protein 2G4 (Cell cycle protein p38-2G4 homolog) (hG4-1) [H.sapiens]
Hs.83532	MCP	membrane cofactor protein (CD46, trophoblast-lymphocyte cross-reactive antigen)
Hs.31210	BCL3	B-cell CLL/lymphoma 3
Hs.96149	NFATC1	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1
Hs.3446	MAP2K1	mitogen-activated protein kinase kinase 1
Hs.68137	ADCYAP1	adenylate cyclase activating polypeptide 1 (pituitary)
Hs.183138	PCOLN3	procollagen (type III) N-endopeptidase
Hs.2014	TRD@	T cell receptor delta locus
Hs.15589	PPARBP	PPAR binding protein
Hs.394	ADM	adrenomedullin
Hs.77502	MAT2A	methionine adenosyltransferase II, alpha
Hs.432833	UBE2I	ubiquitin-conjugating enzyme E2I (UBC9 homolog, yeast)
Hs.96	PMAIP1	phorbol-12-myristate-13-acetate-induced protein 1
Hs.90875	RABIF	RAB interacting factor
Hs.181301	CTSS	cathepsin S
Hs.77490	GSTT1	glutathione S-transferase theta 1
Hs.94479	TMEM1	transmembrane protein 1
Hs.15265	HNRPR	heterogeneous nuclear ribonucleoprotein R
Hs.80658	UCP2	uncoupling protein 2 (mitochondrial, proton carrier)
Hs.183153	ARF4L	ADP-ribosylation factor 4-like
Hs.83974	SLC21A2	solute carrier family 21 (prostaglandin transporter), member 2
Hs.82643	PTK9	protein tyrosine kinase 9
Hs.181289	ELA3A	elastase 3A, pancreatic (protease E)
Hs.75206	PPP3CC	protein phosphatase 3 (formerly 2B), catalytic subunit, gamma isoform (calcineurin A gamma)
Hs.13572	CAMLG	calcium modulating ligand
Hs.78867	PTPRZ1	protein tyrosine phosphatase, receptor-type, Z polypeptide 1
Hs.43388	IFRG28	28kD interferon responsive protein
Hs.180877	H3F3B	H3 histone, family 3B (H3.3B)
Hs.861	MAPK3	mitogen-activated protein kinase 3
Hs.423615	CDC34	cell division cycle 34
Hs.1790	NR3C2	nuclear receptor subfamily 3, group C, member 2
Hs.432811	DGUOK	deoxyguanosine kinase
Hs.89499	ALOX5	arachidonate 5-lipoxygenase
Hs.71592	AA171715	Homo sapiens cDNA: FLJ21893 fis, clone HEP03412, mRNA sequence
Hs.277477	HLA-C	major histocompatibility complex, class I, C
Hs.374993	AA076063	Homo sapiens, clone IMAGE:4296901, mRNA, mRNA sequence
Hs.183773	GOLGA4	golgi autoantigen, golgin subfamily a, 4
Hs.150477	WRN	Werner syndrome
Hs.687	CYP4B1	cytochrome P450, subfamily IVB, polypeptide 1
Hs.80741	PCCA	propionyl Coenzyme A carboxylase, alpha polypeptide
Hs.154084	PYGM	phosphorylase, glycogen; muscle (McArdle syndrome, glycogen storage disease type V)

Hs.182255	NHP2L1	NHP2 non-histone chromosome protein 2-like 1 (S. cerevisiae)
Hs.38125	SP110	SP110 nuclear body protein
Hs.74576	GDI1	GDP dissociation inhibitor 1
Hs.400740	PPP2R4	protein phosphatase 2A, regulatory subunit B' (PR 53)
Hs.10803	CIB1	calcium and integrin binding 1 (calmyrin)
Hs.74647	TRA@	T cell receptor alpha locus
Hs.94672	GCN5L1	GCN5 general control of amino-acid synthesis 5-like 1 (yeast)
Hs.74451	CAPNS1	calpain, small subunit 1
Hs.153053	CD37	CD37 antigen
Hs.82587	PLD1	phospholipase D1, phophatidylcholine-specific
Hs.21254	TRIP	TRAF interacting protein
Hs.75207	GLO1	glyoxalase I
Hs.104925	ENC1	ectodermal-neural cortex (with BTB-like domain)
Hs.372732	EB-1	E2a-Pbx1-associated protein
Hs.78853	UNG	uracil-DNA glycosylase
Hs.75290	ARF4	ADP-ribosylation factor 4
Hs.78864	FCGR2A	Fc fragment of IgG, low affinity IIa, receptor for (CD32)
Hs.85092	TRIP11	thyroid hormone receptor interactor 11
Hs.20084	RXRA	retinoid X receptor, alpha
Hs.40968	HS3ST1	heparan sulfate (glucosamine) 3-O-sulfotransferase 1
Hs.14894	TGOLN2	trans-golgi network protein 2
Hs.90077	TGIF	TGFB-induced factor (TALE family homeobox)
Hs.90791	GABRA6	gamma-aminobutyric acid (GABA) A receptor, alpha 6
Hs.279929	HSGP25L2G	gp25L2 protein
Hs.236361	RNPC1	RNA-binding region (RNP1, RRM) containing 1
Hs.93837	PITPNM	phosphatidylinositol transfer protein, membrane-associated
Hs.77837	UGP2	UDP-glucose pyrophosphorylase 2
Hs.380728	SLC31A1	solute carrier family 31 (copper transporters), member 1
Hs.13340	HAT1	histone acetyltransferase 1
Hs.77522	HLA-DMA	major histocompatibility complex, class II, DM alpha
Hs.89691	UGT2B4	UDP glycosyltransferase 2 family, polypeptide B4
Hs.372664	OVCA2	candidate tumor suppressor OVCA2
Hs.77054	BTG1	B-cell translocation gene 1, anti-proliferative
Hs.1652	CCR7	chemokine (C-C motif) receptor 7
Hs.9908	NIFU	nitrogen fixation cluster-like
Hs.162808	PIK3CD	phosphoinositide-3-kinase, catalytic, delta polypeptide
Hs.82128	TPBG	trophoblast glycoprotein
Hs.77667	LY6E	lymphocyte antigen 6 complex, locus E
Hs.225977	NCOA3	nuclear receptor coactivator 3
Hs.75812	PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial)
Hs.82028	TGFBR2	transforming growth factor, beta receptor II (70/80kDa)
Hs.1166	THPO	thrombopoietin (myeloproliferative leukemia virus oncogene ligand, megakaryocyte growth and development factor)
Hs.260523	NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog
Hs.315463	IL24	interleukin 24
Hs.19192	CDK2	cyclin-dependent kinase 2
Hs.180655	STK12	serine/threonine kinase 12
Hs.74267	RPL15	ribosomal protein L15
Hs.2230	EGF	epidermal growth factor (beta-urogastrone)
Hs.301819	ZNF146	zinc finger protein 146
Hs.1313	TNFSF8	tumor necrosis factor (ligand) superfamily, member 8
Hs.239307	YARS	tyrosyl-tRNA synthetase
Hs.80828	KRT1	keratin 1 (epidermolytic hyperkeratosis)
Hs.100293	OGT	O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase)
Hs.422789	VRK1	vaccinia related kinase 1
Hs.77558	HMGN3	high mobility group nucleosomal binding domain 3
Hs.184488	FLOT2	flotillin 2

Hs.82749	TM4SF2	transmembrane 4 superfamily member 2
Hs.154782	AP3S2	adaptor-related protein complex 3, sigma 2 subunit
Hs.33642	ARCN1	archain 1
Hs.82916	CCT6A	chaperonin containing TCP1, subunit 6A (zeta 1)
Hs.109051	SH3BGRL3	SH3 domain binding glutamic acid-rich protein like 3
Hs.303157	TRB@	T cell receptor beta locus
Hs.96149	NFATC1	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1
Hs.79357	PSMC6	proteasome (prosome, macropain) 26S subunit, ATPase, 6

Appendix 6

6.1 Table of genes with significantly decreased total RNA levels in B-CLL cells, when compared to tonsil CD19+ cells.

Cluster Number	Gene Name	Gene Description
Hs.64794	ZNF183	zinc finger protein 183 (RING finger, C3HC4 type)
Hs.290070	GSN	gelsolin (amyloidosis, Finnish type)
Hs.75617	COL4A2	collagen, type IV, alpha 2
Hs.321164	NPAS2	neuronal PAS domain protein 2
Hs.166684	STK3	serine/threonine kinase 3 (STE20 homolog, yeast)
Hs.79404	D4S234E	DNA segment on chromosome 4 (unique) 234 expressed sequence
Hs.30054	F5	coagulation factor V (proaccelerin, labile factor)
Hs.37616	MGC14480	hypothetical protein MGC14480
Hs.24129	FLJ10716	CLLL7 protein
Hs.265827	G1P3	interferon, alpha-inducible protein (clone IFI-6-16)
Hs.15154	SRPX	sushi-repeat-containing protein, X chromosome
Hs.421376	H85749	ESTs, Highly similar to deleted in split-hand/split-foot 1 region [Homo sapiens] [H.sapiens]
Hs.381099	LCP1	lymphocyte cytosolic protein 1 (L-plastin)
Hs.82193	ESD	esterase D/formylglutathione hydrolase
Hs.250	XDH	xanthene dehydrogenase
Hs.89578	GTF2H1	general transcription factor IIH, polypeptide 1, 62kDa
Hs.367740	HSPC022	HSPC022 protein
Hs.66713	DIPA	hepatitis delta antigen-interacting protein A
Hs.180414	HSPA8	heat shock 70kDa protein 8
Hs.75124	CDR2	cerebellar degeneration-related protein 2, 62kDa
Hs.91813	BTN2A2	butyrophilin, subfamily 2, member A2
Hs.18212	DXS9879E	DNA segment on chromosome X (unique) 9879 expressed sequence
Hs.70725	GABRP	gamma-aminobutyric acid (GABA) A receptor, pi
Hs.324696	KIAA1594	KIAA1594 protein
Hs.77929	ERCC3	excision repair cross-complementing rodent repair deficiency, complementation group 3 (xeroderma pigmentosum group B complementing)
Hs.86724	GCH1	GTP cyclohydrolase 1 (dopa-responsive dystonia)
Hs.31622	CNTNAP1	contactin associated protein 1
Hs.111301	MMP2	matrix metalloproteinase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)
Hs.23179	SYT5	synaptotagmin V
Hs.404	MLLT3	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 3
Hs.207776	AGA	aspartylglucosaminidase
Hs.167791	RCN1	reticulocalbin 1, EF-hand calcium binding domain
Hs.182741	TIAL1	TIA1 cytotoxic granule-associated RNA binding protein-like 1
Hs.122843	CASP8AP2	CASP8 associated protein 2
Hs.30956	NHLH1	nescient helix loop helix 1
Hs.75339	INPPL1	inositol polyphosphate phosphatase-like 1
Hs.343575	ABI-2	abl-interactor 2
Hs.406579	H11482	ESTs, Highly similar to INGR_HUMAN Interferon-gamma receptor alpha chain precursor (CDw119) [H.sapiens]
Hs.396877	H71847	ESTs
Hs.51	PIGA	phosphatidylinositol glycan, class A (paroxysmal nocturnal hemoglobinuria)

Hs.376220	AA291995	Homo sapiens cDNA FLJ38365 fis, clone FEBRA2001012, highly similar to CLEAVAGE STIMULATION FACTOR, 64 KDA SUBUNIT, mRNA sequence
Hs.84264	ANP32B	acidic (leucine-rich) nuclear phosphoprotein 32 family, member B
Hs.251064	HMGN1	high-mobility group nucleosome binding domain 1
Hs.303649	CCL2	chemokine (C-C motif) ligand 2
Hs.83393	CST6	cystatin E/M
Hs.1581	GSTT2	glutathione S-transferase theta 2
Hs.272429	CASR	calcium-sensing receptor (hypocalciuric hypercalcemia 1, severe neonatal hyperparathyroidism)
Hs.76325	IGJ	immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides
Hs.709	DCK	deoxycytidine kinase
Hs.44222	CGI-90	CGI-90 protein
Hs.82483	MADH2	MAD, mothers against decapentaplegic homolog 2 (Drosophila)
Hs.80988	COL6A3	collagen, type VI, alpha 3
Hs.1742	IQGAP1	IQ motif containing GTPase activating protein 1
Hs.356317	RPS21	ribosomal protein S21
Hs.146688	PTGES	prostaglandin E synthase
Hs.75319	RRM2	ribonucleotide reductase M2 polypeptide
Hs.31472	MAP3K7IP1	mitogen-activated protein kinase kinase kinase 7 interacting protein 1
Hs.2795	LDHA	lactate dehydrogenase A
Hs.154495	ACHE	acetylcholinesterase (YT blood group)
Hs.250899	HSBP1	heat shock factor binding protein 1
Hs.76392	ALDH1A1	aldehyde dehydrogenase 1 family, member A1
Hs.433103	AI479768	ESTs, Highly similar to A54821 apoptosis regulator ICH-1, stimulatory form L - human [H.sapiens]
Hs.108623	THBS2	thrombospondin 2
Hs.75256	RGS1	regulator of G-protein signalling 1
Hs.278526	RNTRE	related to the N terminus of tre
Hs.9700	CCNE1	cyclin E1
Hs.79889	MMD	monocyte to macrophage differentiation-associated
Hs.2352	ADCY2	adenylate cyclase 2 (brain)
Hs.81687	NME3	non-metastatic cells 3, protein expressed in
Hs.154879	DGCR14	DiGeorge syndrome critical region gene 14
Hs.83918	AMPD3	adenosine monophosphate deaminase (isoform E)
Hs.78601	UROD	uroporphyrinogen decarboxylase
Hs.93523	PPIL2	peptidylprolyl isomerase (cyclophilin)-like 2
Hs.78353	SRPK2	SFRS protein kinase 2
Hs.819	HOXB7	homeo box B7
Hs.99948	BMP8	bone morphogenetic protein 8 (osteogenic protein 2)
Hs.98658	BUB1	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)
Hs.16297	COX17	COX17 homolog, cytochrome c oxidase assembly protein (yeast)
Hs.144567	AGXT	alanine-glyoxylate aminotransferase (oxalosis I; hyperoxaluria I; glycolicaciduria; serine-pyruvate aminotransferase)
Hs.75799	PRSS8	protease, serine, 8 (prostasin)
Hs.81469	NUBP1	nucleotide binding protein 1 (MinD homolog, E. coli)
Hs.169886	TNXB	tenascin XB
Hs.179657	PLAUR	plasminogen activator, urokinase receptor
Hs.220689	G3BP	Ras-GTPase-activating protein SH3-domain-binding protein
Hs.81634	ATP5F1	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b, isoform 1
Hs.92137	R62862	ESTs, Highly similar to MYCL_HUMAN L-myc-1 proto-oncogene protein [H.sapiens]
Hs.66	IL1RL1	interleukin 1 receptor-like 1
Hs.77513	COX10	COX10 homolog, cytochrome c oxidase assembly protein, heme A: farnesyltransferase (yeast)

Hs.35	PTPN7	protein tyrosine phosphatase, non-receptor type 7
Hs.181107	ANXA13	annexin A13
Hs.430924	AA670438	EST, Weakly similar to UBL1_HUMAN Ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCH-L1) (Ubiquitin thiolesterase L1) (Neuron cytoplasmic protein 9.5) (PGP 9.5) (PGP9.5) [H.sapiens]
Hs.178237	TH	tyrosine hydroxylase
Hs.146847	TANK	TRAF family member-associated NFKB activator
Hs.334330	CALM3	calmodulin 3 (phosphorylase kinase, delta)
Hs.73073	ANKRD7	ankyrin repeat domain 7
Hs.74124	OA1	ocular albinism 1 (Nettleship-Falls)
Hs.78793	PRKCZ	protein kinase C, zeta
Hs.105806	GNLY	granulysin
Hs.347508	BG185544	Homo sapiens cDNA: FLJ23482 fis, clone KAIA03142, mRNA sequence
Hs.350265	PRSS15	protease, serine, 15
Hs.20894	NDST1	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1
Hs.10758	NDUFS4	NADH dehydrogenase (ubiquinone) Fe-S protein 4, 18kDa (NADH-coenzyme Q reductase)
Hs.3844	LMO4	LIM domain only 4
Hs.12482	GNPAT	glyceronephosphate O-acyltransferase
Hs.24976	ART3	ADP-ribosyltransferase 3
Hs.155455	PFKL	phosphofructokinase, liver
Hs.170250	C4A	complement component 4A
Hs.78436	EPHB1	EphB1
Hs.77462	DNMT1	DNA (cytosine-5-)-methyltransferase 1
Hs.8700	DLC1	deleted in liver cancer 1
Hs.287995	AA136271	Homo sapiens cDNA: FLJ23181 fis, clone LNG11094, mRNA sequence
Hs.178281	BRDT	bromodomain, testis-specific
Hs.89717	CPA2	carboxypeptidase A2 (pancreatic)
Hs.146388	MAP7	microtubule-associated protein 7
Hs.79361	KLK6	kallikrein 6 (neurosin, zyme)
Hs.66052	CD38	CD38 antigen (p45)
Hs.414985	H56265	ESTs, Highly similar to GSH1_HUMAN Glutamate--cysteine ligase catalytic subunit (Gamma-glutamylcysteine synthetase) (Gamma-ECS) (GCS heavy chain) [H.sapiens]
Hs.378711	AA001443	Homo sapiens cDNA FLJ40855 fis, clone TRACH2016317, highly similar to HOMEOBOX PROTEIN MEIS1, mRNA sequence
Hs.343628	SIAT4B	sialyltransferase 4B (beta-galactoside alpha-2,3-sialytransferase)
Hs.75105	EBP	emopamil binding protein (sterol isomerase)
Hs.90443	NDUFS8	NADH dehydrogenase (ubiquinone) Fe-S protein 8, 23kDa (NADH-coenzyme Q reductase)
Hs.7857	EPB41L2	erythrocyte membrane protein band 4.1-like 2
Hs.199248	PTGER4	prostaglandin E receptor 4 (subtype EP4)
Hs.279919	RBX1	ring-box 1
Hs.12553	R39258	ESTs
Hs.377912	CYP11B1	cytochrome P450, subfamily XIB (steroid 11-beta-hydroxylase), polypeptide 1
Hs.381154	TPMT	thiopurine S-methyltransferase
Hs.283738	CSNK1A1	casein kinase 1, alpha 1
Hs.76873	HYAL2	hyaluronoglucosaminidase 2
Hs.13046	TXNRD1	thioredoxin reductase 1
Hs.75212	ODC1	ornithine decarboxylase 1
Hs.184222	DSCR1	Down syndrome critical region gene 1
Hs.89433	ABCC1	ATP-binding cassette, sub-family C (CFTR/MRP), member 1
Hs.372651	MGC29643	hypothetical protein MGC29643
Hs.597	GOT1	glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1)

Hs.186809	AA700647	ESTs, Highly similar to leukocyte cell-derived chemotaxin 2 [Homo sapiens] [H.sapiens]
Hs.4756	FEN1	flap structure-specific endonuclease 1
Hs.80684	HMGB2	high-mobility group box 2
Hs.78466	PSMD8	proteasome (prosome, macropain) 26S subunit, non-ATPase, 8
Hs.79095	EPS15	epidermal growth factor receptor pathway substrate 15
Hs.78996	PCNA	proliferating cell nuclear antigen
Hs.49007	PAPOLA	poly(A) polymerase alpha
Hs.519	WWOX	WW domain containing oxidoreductase
Hs.64016	PROS1	protein S (alpha)
Hs.121509	COL11A2	collagen, type XI, alpha 2
Hs.198862	FBLN2	fibulin 2
Hs.2074	ZFX	zinc finger protein, X-linked
Hs.75318	TUBA1	tubulin, alpha 1 (testis specific)
Hs.154151	PTPRM	protein tyrosine phosphatase, receptor type, M
Hs.154443	MCM4	MCM4 minichromosome maintenance deficient 4 (S. cerevisiae)
Hs.101408	BCAT2	branched chain aminotransferase 2, mitochondrial
Hs.29882	FAM3C	family with sequence similarity 3, member C
Hs.150403	DDC	dopa decarboxylase (aromatic L-amino acid decarboxylase)
Hs.343564	TBCE	tubulin-specific chaperone e
Hs.33532	ZNF151	zinc finger protein 151 (pHZ-67)
Hs.250870	MAP2K5	mitogen-activated protein kinase kinase 5
Hs.2288	VSNL1	visinin-like 1
Hs.84318	RPA1	replication protein A1, 70kDa
Hs.82401	CD69	CD69 antigen (p60, early T-cell activation antigen)
Hs.170917	PTGER3	prostaglandin E receptor 3 (subtype EP3)
Hs.79300	UBE2V2	ubiquitin-conjugating enzyme E2 variant 2
Hs.585	APOB	apolipoprotein B (including Ag(x) antigen)
Hs.419776	AA167269	Homo sapiens cDNA FLJ11689 fis, clone HEMBA1004977, mRNA sequence
Hs.425944	T57841	EST, Highly similar to UFD1_HUMAN Ubiquitin fusion degradation protein 1 homolog (UB fusion protein 1) [H.sapiens]
Hs.2022	TGM3	transglutaminase 3 (E polypeptide, protein-glutamine-gamma-glutamyltransferase)
Hs.154138	CHI3L2	chitinase 3-like 2
Hs.46362	HTR2C	5-hydroxytryptamine (serotonin) receptor 2C
Hs.82794	CETN2	centrin, EF-hand protein, 2
Hs.75761	SRPK1	SFRS protein kinase 1
Hs.252876	MMP23A	matrix metalloproteinase 23A
Hs.165843	CSNK2B	casein kinase 2, beta polypeptide
Hs.2420	SOD3	superoxide dismutase 3, extracellular
Hs.343586	ZFP36	zinc finger protein 36, C3H type, homolog (mouse)
Hs.287827	R53330	ESTs, Highly similar to MDR3_HUMAN Multidrug resistance protein 3 (P-glycoprotein 3) [H.sapiens]
Hs.76913	PSMA5	proteasome (prosome, macropain) subunit, alpha type, 5
Hs.75569	RELA	v-rel reticuloendotheliosis viral oncogene homolog A, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3, p65 (avian)
Hs.87149	ITGB3	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
Hs.169756	C1S	complement component 1, s subcomponent
Hs.82906	CDC20	CDC20 cell division cycle 20 homolog (S. cerevisiae)
Hs.239663	MLLT7	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 7
Hs.352341	STCH	stress 70 protein chaperone, microsome-associated, 60kDa
Hs.171595	HTATSF1	HIV TAT specific factor 1
Hs.82212	CD53	CD53 antigen
Hs.576	FUCA1	fucosidase, alpha-L- 1, tissue
Hs.78712	ALAS1	aminolevulinate, delta-, synthase 1
Hs.106415	PPARD	peroxisome proliferative activated receptor, delta

Hs.75196	BAT8	HLA-B associated transcript 8
Hs.78	GABPA	GA binding protein transcription factor, alpha subunit 60kDa
Hs.241570	TNF	tumor necrosis factor (TNF superfamily, member 2)
Hs.36	LTA	lymphotoxin alpha (TNF superfamily, member 1)
Hs.93913	IL6	interleukin 6 (interferon, beta 2)
Hs.298184	KCNAB2	potassium voltage-gated channel, shaker-related subfamily, beta member 2
Hs.29475	TYMS	thymidylate synthetase
Hs.171495	RARB	retinoic acid receptor, beta
Hs.234569	ZAP70	zeta-chain (TCR) associated protein kinase 70kDa
Hs.193124	PDK3	pyruvate dehydrogenase kinase, isoenzyme 3
Hs.169476	GAPD	glyceraldehyde-3-phosphate dehydrogenase
Hs.335918	FDPS	farnesyl diphosphate synthase (farnesyl pyrophosphate synthetase, dimethylallyltranstransferase, geranyltranstransferase)
Hs.77432	EGFR	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
Hs.75428	SOD1	superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))
Hs.114599	COL8A1	collagen, type VIII, alpha 1
Hs.82116	MYD88	myeloid differentiation primary response gene (88)
Hs.840	INDO	indoleamine-pyrrole 2,3 dioxygenase
Hs.270833	AREG	amphiregulin (schwannoma-derived growth factor)
Hs.152931	LBR	lamin B receptor
Hs.356386	RAB7	RAB7, member RAS oncogene family
Hs.373522	NRAP	nebulin-related anchoring protein
Hs.83469	NFE2L1	nuclear factor (erythroid-derived 2)-like 1
Hs.394389	PPIB	peptidylprolyl isomerase B (cyclophilin B)
Hs.296634	CP	ceruloplasmin (ferroxidase)
Hs.336916	DAXX	death-associated protein 6
Hs.332173	TLE2	transducin-like enhancer of split 2 (E(sp1) homolog, Drosophila)
Hs.80552	DPT	dermatopontin
Hs.334450	IRF5	interferon regulatory factor 5
Hs.422892	N57964	EST
Hs.83656	ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta
Hs.433506	AA188155	Homo sapiens, clone IMAGE:3457786, mRNA, mRNA sequence
Hs.78943	BLMH	bleomycin hydrolase
Hs.284244	FGF2	fibroblast growth factor 2 (basic)
Hs.75474	NPHP1	nephronophthisis 1 (juvenile)
Hs.4	ADH1B	alcohol dehydrogenase IB (class I), beta polypeptide
Hs.117367	SLC22A1	solute carrier family 22 (organic cation transporter), member 1
Hs.46423	H4FG	H4 histone family, member G
Hs.75789	NDRG1	N-myc downstream regulated gene 1
Hs.78881	MEF2B	MADS box transcription enhancer factor 2, polypeptide B (myocyte enhancer factor 2B)
Hs.1519	PRKAR1B	protein kinase, cAMP-dependent, regulatory, type I, beta
Hs.2507	HTR2B	5-hydroxytryptamine (serotonin) receptor 2B
Hs.180930	BTAF1	BTAF1 RNA polymerase II, B-TFIID transcription factor-associated, 170kDa (Mot1 homolog, S. cerevisiae)
Hs.84232	TCN2	transcobalamin II; macrocytic anemia
Hs.348935	IGLL1	immunoglobulin lambda-like polypeptide 1
Hs.74107	ZNF43	zinc finger protein 43 (HTF6)
Hs.135626	CMA1	chymase 1, mast cell
Hs.96398	OGG1	8-oxoguanine DNA glycosylase
Hs.180866	IFNGR1	interferon gamma receptor 1
Hs.737	ETR101	immediate early protein
Hs.171280	HADH2	hydroxyacyl-Coenzyme A dehydrogenase, type II
Hs.189999	P2Y5	purinergic receptor (family A group 5)
Hs.160958	CDC37	CDC37 cell division cycle 37 homolog (S. cerevisiae)

Hs.433790	AA699876	ESTs, Moderately similar to putative peroxisome microbody protein 175.1 [Homo sapiens] [H.sapiens]
Hs.239069	FHL1	four and a half LIM domains 1
Hs.179565	MCM3	MCM3 minichromosome maintenance deficient 3 (S. cerevisiae)
Hs.423054	N69689	EST
Hs.326035	EGR1	early growth response 1
Hs.170027	MDM2	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)
Hs.406161	H90348	ESTs, Highly similar to PCL1_HUMAN Prenylcysteine lyase precursor [H.sapiens]
Hs.433394	TUBA3	tubulin, alpha 3
Hs.370622	W90381	Homo sapiens clone R19540 SNURF-SNRPN mRNA, downstream untranslated exons, alternatively spliced, mRNA sequence
Hs.169919	ETFA	electron-transfer-flavoprotein, alpha polypeptide (glutaric aciduria II)
Hs.95990	PKLR	pyruvate kinase, liver and RBC
Hs.8074	BAI3	brain-specific angiogenesis inhibitor 3
Hs.25482	EVPL	envoplakin
Hs.119403	HEXA	hexosaminidase A (alpha polypeptide)
Hs.72916	GLI3	GLI-Kruppel family member GLI3 (Greig cephalopolysyndactyly syndrome)
Hs.170019	RUNX3	runt-related transcription factor 3
Hs.184601	SLC7A5	solute carrier family 7 (cationic amino acid transporter, y+ system), member 5
Hs.356785	AA193254	ESTs, Highly similar to IF4E_HUMAN Eukaryotic translation initiation factor 4E (eIF-4E) (eIF4E) (mRNA cap-binding protein) (eIF-4F 25 kDa subunit) [H.sapiens]
Hs.58435	FYB	FYN binding protein (FYB-120/130)
Hs.75752	COX7B	cytochrome c oxidase subunit VIIb
Hs.1973	CCNF	cyclin F
Hs.40300	CAPN3	calpain 3, (p94)
Hs.101657	AA487543	ESTs, Highly similar to cysteine and histidine-rich domain (CHORD)-containing, zinc-binding protein 1; chord domain-containing protein 1 [Homo sapiens] [H.sapiens]
Hs.82733	NID2	nidogen 2 (osteonidogen)
Hs.14601	HCLS1	hematopoietic cell-specific Lyn substrate 1
Hs.82927	AMPD2	adenosine monophosphate deaminase 2 (isoform L)
Hs.79339	LGALS3BP	lectin, galactoside-binding, soluble, 3 binding protein
Hs.27424	DDX11	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL1-like helicase homolog, S. cerevisiae)
Hs.89695	INSR	insulin receptor
Hs.179661	AA427899	Beta-tubulin [Homo sapiens], mRNA sequence
Hs.433707	HLF	hepatic leukemia factor
Hs.406384	CBX3	chromobox homolog 3 (HP1 gamma homolog, Drosophila)
Hs.197540	HIF1A	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)
Hs.79090	XPO1	exportin 1 (CRM1 homolog, yeast)
Hs.3416	ADFP	adipose differentiation-related protein
Hs.38069	C8B	complement component 8, beta polypeptide
Hs.432314	AA431967	Homo sapiens, clone IMAGE:4816693, mRNA, mRNA sequence
Hs.14623	IFI30	interferon, gamma-inducible protein 30
Hs.6467	SYNGR3	synaptogyrin 3
Hs.150423	CDK9	cyclin-dependent kinase 9 (CDC2-related kinase)
Hs.349650	RAF1	v-raf-1 murine leukemia viral oncogene homolog 1
Hs.170040	PDGFRL	platelet-derived growth factor receptor-like
Hs.433506	AA188155	Homo sapiens, clone IMAGE:3457786, mRNA, mRNA sequence
Hs.102950	IGLJ3	immunoglobulin lambda joining 3
Hs.147097	H2AFX	H2A histone family, member X
Hs.433618	MLH1	mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli)

Hs.1861	MPP1	membrane protein, palmitoylated 1, 55kDa
Hs.9884	TUBGCP3	tubulin, gamma complex associated protein 3
Hs.1540	THOC1	THO complex 1
Hs.172471	KCNAB1	potassium voltage-gated channel, shaker-related subfamily, beta member 1
Hs.80288	HSPA1L	heat shock 70kDa protein 1-like
Hs.109606	CORO1A	coronin, actin binding protein, 1A
Hs.433955	FLJ20886	hypothetical protein FLJ20886
Hs.251653	TUBB2	tubulin, beta, 2
Hs.82237	TRIM29	tripartite motif-containing 29
Hs.211608	NUP153	nucleoporin 153kDa
Hs.119597	SCD	stearoyl-CoA desaturase (delta-9-desaturase)
Hs.300697	IGHG3	immunoglobulin heavy constant gamma 3 (G3m marker)
Hs.149443	101F6	putative tumor suppressor 101F6
Hs.180714	COX6A1	cytochrome c oxidase subunit VIa polypeptide 1
Hs.389137	MFAP2	microfibrillar-associated protein 2
Hs.355722	ITGB8	integrin, beta 8
Hs.79350	RYK	RYK receptor-like tyrosine kinase
Hs.41185	FLJ40021	hypothetical protein FLJ40021
Hs.3123	LLGL2	lethal giant larvae homolog 2 (Drosophila)
Hs.150956	EXTL1	exostoses (multiple)-like 1
Hs.279474	MKRN2	makorin, ring finger protein, 2
Hs.90011	ADSS	adenylosuccinate synthase
Hs.2877	CDH3	cadherin 3, type 1, P-cadherin (placental)
Hs.196209	RAE1	RAE1 RNA export 1 homolog (S. pombe)
Hs.100322	CA6	carbonic anhydrase VI
Hs.192570	FLJ22028	hypothetical protein FLJ22028
Hs.2142	HTR3A	5-hydroxytryptamine (serotonin) receptor 3A
Hs.15110	ZNF211	zinc finger protein 211
Hs.184585	LMO2	LIM domain only 2 (rhombotin-like 1)
Hs.376516	AA424675	Homo sapiens cDNA FLJ35825 fis, clone TESTI2006360, mRNA sequence
Hs.74621	PRNP	prion protein (p27-30) (Creutzfeld-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia)
Hs.16269	BCL7B	B-cell CLL/lymphoma 7B
Hs.83758	CKS2	CDC28 protein kinase regulatory subunit 2
Hs.76240	AK1	adenylate kinase 1
Hs.367667	PMS2L8	postmeiotic segregation increased 2-like 8
Hs.117572	CCBP2	chemokine binding protein 2
Hs.2233	CSF3	colony stimulating factor 3 (granulocyte)
Hs.75551	RSU1	Ras suppressor protein 1
Hs.79516	BASP1	brain abundant, membrane attached signal protein 1
Hs.155140	CSNK2A1	casein kinase 2, alpha 1 polypeptide
Hs.278311	PLXNB1	plexin B1
Hs.979	PDHB	pyruvate dehydrogenase (lipoamide) beta
Hs.100469	MLLT4	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 4
Hs.429	ATP5G3	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9) isoform 3
Hs.5120	DNCL1	dynein, cytoplasmic, light polypeptide 1
Hs.170157	MYO5A	myosin VA (heavy polypeptide 12, myoxin)
Hs.79172	SLC25A5	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5
Hs.20830	KNSL2	kinesin-like 2
Hs.80424	F13A1	coagulation factor XIII, A1 polypeptide
Hs.84520	YAP1	Yes-associated protein 1, 65kDa
Hs.42957	METTL1	methyltransferase-like 1
Hs.180248	ZNF124	zinc finger protein 124 (HZF-16)

Hs.356463	FNTA	farnesyltransferase, CAAX box, alpha
Hs.272493	CCL15	chemokine (C-C motif) ligand 15
Hs.410626	AI363781	EST
Hs.150917	CTNNA2	catenin (cadherin-associated protein), alpha 2
Hs.20225	TFIP11	tuftelin interacting protein 11
Hs.75334	EXT2	exostoses (multiple) 2
Hs.367689	TRIO	triple functional domain (PTPRF interacting)
Hs.172210	MUF1	MUF1 protein
Hs.349416	MOCS1	molybdenum cofactor synthesis 1
Hs.170027	MDM2	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)
Hs.16695	UBE1L	ubiquitin-activating enzyme E1-like
Hs.204732	MMP26	matrix metalloproteinase 26
Hs.38084	SULT1C1	sulfotransferase family, cytosolic, 1C, member 1
Hs.78935	METAP2	methionyl aminopeptidase 2
Hs.75268	SIAT4C	sialyltransferase 4C (beta-galactoside alpha-2,3-sialytransferase)
Hs.1376	HSD11B2	hydroxysteroid (11-beta) dehydrogenase 2
Hs.324784	GAD1	glutamate decarboxylase 1 (brain, 67kDa)
Hs.78068	CPZ	carboxypeptidase Z
Hs.434043	AA430574	ESTs, Highly similar to A55933 paxillin - human [H.sapiens]
Hs.278568	HFL1	H factor (complement)-like 1
Hs.233952	PSMA7	proteasome (prosome, macropain) subunit, alpha type, 7
Hs.64794	ZNF183	zinc finger protein 183 (RING finger, C3HC4 type)

Appendix 7

7.1 Table of genes with up-regulated transcription and translation

Cluster Number	Gene Name	Gene Description
Hs.80658	UCP2	uncoupling protein 2 (mitochondrial, proton carrier)
Hs.80423	PBP	prostatic binding protein
Hs.89472	AGTR1	angiotensin II receptor, type 1
Hs.315463	IL24	interleukin 24
Hs.77054	BTG1	B-cell translocation gene 1, anti-proliferative
Hs.258503	CYFIP2	cytoplasmic FMR1 interacting protein 2
Hs.183773	GOLGA4	golgi autoantigen, golgin subfamily a, 4
Hs.1790	NR3C2	nuclear receptor subfamily 3, group C, member 2
Hs.277477	HLA-C	major histocompatibility complex, class I, C
Hs.38125	SP110	SP110 nuclear body protein
Hs.103042	MAP1B	microtubule-associated protein 1B
Hs.56937	ST14	suppression of tumorigenicity 14 (colon carcinoma, matriptase, epithin)
Hs.75149	SH3GL2	SH3-domain GRB2-like 2
Hs.180877	H3F3B	H3 histone, family 3B (H3.3B)
Hs.33642	ARCN1	archain 1

7.2 Table of genes with down-regulated transcription and translation

Cluster Number	Gene Name	Gene Description
Hs.20894	NDST1	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1
Hs.95990	PKLR	pyruvate kinase, liver and RBC
Hs.2420	SOD3	superoxide dismutase 3, extracellular
Hs.29475	TYMS	thymidylate synthetase
Hs.4756	FEN1	flap structure-specific endonuclease 1
Hs.16269	BCL7B	B-cell CLL/lymphoma 7B
Hs.75318	TUBA1	tubulin, alpha 1 (testis specific)
Hs.251653	TUBB2	tubulin, beta, 2
Hs.166684	STK3	serine/threonine kinase 3 (STE20 homolog, yeast)
Hs.14623	IFI30	interferon, gamma-inducible protein 30
Hs.46423	H4FG	H4 histone family, member G
Hs.433506	AA188155	Homo sapiens, clone IMAGE:3457786, mRNA, mRNA sequence
Hs.1376	HSD11B2	hydroxysteroid (11-beta) dehydrogenase 2
Hs.76325	IGJ	immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides
Hs.40300	CAPN3	calpain 3, (p94)
Hs.92137	R62862	ESTs, Highly similar to MYCL_HUMAN L-myc-1 proto-oncogene protein [H.sapiens]
Hs.233952	PSMA7	proteasome (prosome, macropain) subunit, alpha type, 7
Hs.1519	PRKAR1B	protein kinase, cAMP-dependent, regulatory, type I, beta
Hs.82483	MADH2	MAD, mothers against decapentaplegic homolog 2 (Drosophila)
Hs.82927	AMPD2	adenosine monophosphate deaminase 2 (isoform L)
Hs.2233	CSF3	colony stimulating factor 3 (granulocyte)
Hs.348935	IGLL1	immunoglobulin lambda-like polypeptide 1
Hs.367689	TRIO	triple functional domain (PTPRF interacting)
Hs.2352	ADCY2	adenylate cyclase 2 (brain)
Hs.737	ETR101	immediate early protein
Hs.64794	ZNF183	zinc finger protein 183 (RING finger, C3HC4 type)
Hs.82401	CD69	CD69 antigen (p60, early T-cell activation antigen)
Hs.300697	IGHG3	immunoglobulin heavy constant gamma 3 (G3m marker)
Hs.347508	BG185544	Homo sapiens cDNA: FLJ23482 fis, clone KAIA03142, mRNA sequence
Hs.102950	IGLJ3	immunoglobulin lambda joining 3
Hs.410626	AI363781	EST
Hs.150917	CTNNA2	catenin (cadherin-associated protein), alpha 2
Hs.75212	ODC1	ornithine decarboxylase 1
Hs.170917	PTGER3	prostaglandin E receptor 3 (subtype EP3)
Hs.24976	ART3	ADP-ribosyltransferase 3
Hs.343575	ABI-2	abl-interactor 2
Hs.519	WWOX	WW domain containing oxidoreductase

7.3 Table of genes with up-regulated transcription but down-regulated translation

Cluster Number	Gene Name	Gene Description
Hs.180655	STK12	serine/threonine kinase 12
Hs.80741	PCCA	propionyl Coenzyme A carboxylase, alpha polypeptide
Hs.93837	PITPNM	phosphatidylinositol transfer protein, membrane-associated
Hs.432833	UBE2I	ubiquitin-conjugating enzyme E2I (UBC9 homolog, yeast)
Hs.74647	TRA@	T cell receptor alpha locus
Hs.183153	ARF4L	ADP-ribosylation factor 4-like
Hs.78853	UNG	uracil-DNA glycosylase
Hs.75969	PROL2	proline rich 2
Hs.17518	cig5	vipirin
Hs.80828	KRT1	keratin 1 (epidermolytic hyperkeratosis)
Hs.79357	PSMC6	proteasome (prosome, macropain) 26S subunit, ATPase, 6

7.3 Table of genes with down-regulated transcription but up-regulated translation

Cluster Number	Gene Name	Gene Description
Hs.356386	RAB7	RAB7, member RAS oncogene family
Hs.78353	SRPK2	SFRS protein kinase 2
Hs.343586	ZFP36	zinc finger protein 36, C3H type, homolog (mouse)
Hs.165843	CSNK2B	casein kinase 2, beta polypeptide
Hs.155455	PFKL	phosphofructokinase, liver
Hs.1540	THOC1	THO complex 1
Hs.840	INDO	indoleamine-pyrrole 2,3 dioxygenase
Hs.89578	GTF2H1	general transcription factor IIH, polypeptide 1, 62kDa
Hs.179565	MCM3	MCM3 minichromosome maintenance deficient 3 (S. cerevisiae)
Hs.83656	ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta

Appendix 8

8.1 Table of genes with higher polysome association in ZAP70 negative patients than in ZAP70 positive patients.

Cluster Number	Gene Name	Gene Description
Hs.62354	LRBA	LPS-responsive vesicle trafficking, beach and anchor containing
Hs.278589	GTF2I	general transcription factor II, i
Hs.75510	ANXA11	annexin A11
Hs.75703	CCL4	chemokine (C-C motif) ligand 4
Hs.75736	APOD	apolipoprotein D
Hs.297753	VIM	vimentin
Hs.79158	ACADM	acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain
Hs.82159	PSMA1	proteasome (prosome, macropain) subunit, alpha type, 1
Hs.374973	PRPF4	PRP4 pre-mRNA processing factor 4 homolog (yeast)
Hs.367762	KRT6A	keratin 6A
Hs.281866	ATP6V1A1	ATPase, H+ transporting, lysosomal 70kDa, V1 subunit A, isoform 1
Hs.709	DCK	deoxycytidine kinase
Hs.272493	CCL15	chemokine (C-C motif) ligand 15
Hs.433201	CDK2AP1	CDK2-associated protein 1
Hs.78465	JUN	v-jun sarcoma virus 17 oncogene homolog (avian)
Hs.78867	PTPRZ1	protein tyrosine phosphatase, receptor-type, Z polypeptide 1
Hs.89472	AGTR1	angiotensin II receptor, type 1
Hs.152818	USP8	ubiquitin specific protease 8
Hs.184326	CDC10	CDC10 cell division cycle 10 homolog (S. cerevisiae)
Hs.8724	STK38	serine/threonine kinase 38
Hs.15384	AP1GBP1	AP1 gamma subunit binding protein 1
Hs.61828	APPBP1	amyloid beta precursor protein binding protein 1, 59kDa
Hs.377973	LMNA	lamin A/C
Hs.37860	KLF1	Kruppel-like factor 1 (erythroid)
Hs.377992	RABGGTA	Rab geranylgeranyltransferase, alpha subunit
Hs.861	MAPK3	mitogen-activated protein kinase 3
Hs.1252	APOH	apolipoprotein H (beta-2-glycoprotein I)
Hs.288433	HNT	neurotrimin
Hs.418740	H18633	Homo sapiens cDNA FLJ39398 fis, clone PLACE6010704, mRNA sequence
Hs.36602	IF	I factor (complement)
Hs.7940	RAP1GDS1	RAP1, GTP-GDP dissociation stimulator 1
Hs.79000	GAP43	growth associated protein 43
Hs.3059	COPB	coatomer protein complex, subunit beta
Hs.267445	MAPK8	mitogen-activated protein kinase 8
Hs.309943	SP140	SP140 nuclear body protein
Hs.250870	MAP2K5	mitogen-activated protein kinase kinase 5
Hs.2934	RRM1	ribonucleotide reductase M1 polypeptide
Hs.183874	CUL4A	cullin 4A
Hs.5120	DNCL1	dynein, cytoplasmic, light polypeptide 1
Hs.300711	ANXA5	annexin A5
Hs.29736	TRAF5	TNF receptor-associated factor 5
Hs.173965	RPS6KA3	ribosomal protein S6 kinase, 90kDa, polypeptide 3
Hs.400275	AA010777	ESTs, Moderately similar to A Chain A, Crystal Structure Of Human Galectin-7 In Complex With Galactose [H.sapiens]
Hs.272529	GPLD1	glycosylphosphatidylinositol specific phospholipase D1
Hs.21189	DNAJA2	DnaJ (Hsp40) homolog, subfamily A, member 2
Hs.250581	SMARCD2	SWI/SNF related, matrix associated, actin dependent regulator of

		chromatin, subfamily d, member 2
Hs.331602	AP1B1	adaptor-related protein complex 1, beta 1 subunit
Hs.350899	AA102454	Homo sapiens cDNA FLJ30484 fis, clone BRAWH2000071, mRNA sequence
Hs.112058	SIVA	CD27-binding (Siva) protein
Hs.79337	PASK	PAS domain containing serine/threonine kinase
Hs.36	LTA	lymphotoxin alpha (TNF superfamily, member 1)
Hs.380096	ACTR3	ARP3 actin-related protein 3 homolog (yeast)
Hs.75812	PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial)
Hs.171880	POLR2A	polymerase (RNA) II (DNA directed) polypeptide A, 220kDa
Hs.155455	PFKL	phosphofructokinase, liver
Hs.2055	UBE1	ubiquitin-activating enzyme E1 (A1S9T and BN75 temperature sensitivity complementing)
Hs.180446	KPNB1	karyopherin (importin) beta 1
Hs.12719	RENT1	regulator of nonsense transcripts 1
Hs.74451	CAPNS1	calpain, small subunit 1
Hs.1741	ITGB7	integrin, beta 7
Hs.159301	IL18R1	interleukin 18 receptor 1
Hs.381099	LCP1	lymphocyte cytosolic protein 1 (L-plastin)
Hs.430561	AA598487	EST, Moderately similar to PUR2_HUMAN Trifunctional purine biosynthetic protein adenosine-3 [Includes: Phosphoribosylamine--glycine ligase (GARS) (Glycinamide ribonucleotide synthetase) (Phosphoribosylglycinamide synthetase); Phosphoribosylformylglycinami
Hs.155530	IFI16	interferon, gamma-inducible protein 16
Hs.2853	PCBP1	poly(rC) binding protein 1
Hs.76507	PIG7	LPS-induced TNF-alpha factor
Hs.78915	GABPB1	GA binding protein transcription factor, beta subunit 1, 53kDa
Hs.333417	CAPZB	capping protein (actin filament) muscle Z-line, beta
Hs.172550	PTBP1	polypyrimidine tract binding protein 1
Hs.2430	TCFL1	transcription factor-like 1
Hs.42945	ASM3A	acid sphingomyelinase-like phosphodiesterase
Hs.318501	TRIM22	tripartite motif-containing 22
Hs.20137	BIKE	BMP-2 inducible kinase
Hs.289114	TNC	tenascin C (hexabrachion)
Hs.74576	GDI1	GDP dissociation inhibitor 1
Hs.77768	DNAJB2	DnaJ (Hsp40) homolog, subfamily B, member 2
Hs.301746	RAP2A	RAP2A, member of RAS oncogene family
Hs.155342	PRKCD	protein kinase C, delta
Hs.12013	ABCE1	ATP-binding cassette, sub-family E (OABP), member 1
Hs.78060	PHKB	phosphorylase kinase, beta
Hs.77617	SP100	nuclear antigen Sp100
Hs.16003	RBBP4	retinoblastoma binding protein 4
Hs.7644	H1F2	H1 histone family, member 2
Hs.196384	PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
Hs.410488	R39356	EST, Weakly similar to hypothetical protein FLJ20489 [Homo sapiens] [H.sapiens]
Hs.108694	GYPA	glycophorin A (includes MN blood group)
Hs.50130	NDN	necdin homolog (mouse)
Hs.166563	RFC1	replication factor C (activator 1) 1, 145kDa
Hs.75253	IDH3G	isocitrate dehydrogenase 3 (NAD+) gamma
Hs.80426	BRE	brain and reproductive organ-expressed (TNFRSF1A modulator)
Hs.79069	AI571464	Homo sapiens mRNA; cDNA DKFZp434B142 (from clone DKFZp434B142), mRNA sequence
Hs.172182	PABPC1	poly(A) binding protein, cytoplasmic 1
Hs.3069	HSPA9B	heat shock 70kDa protein 9B (mortalin-2)
Hs.91813	BTN2A2	butyrophilin, subfamily 2, member A2

Hs.154295	SP3	Sp3 transcription factor
Hs.169139	KYNU	kynureninase (L-kynurenine hydrolase)
Hs.2006	GSTM3	glutathione S-transferase M3 (brain)
Hs.183123	NR5A2	nuclear receptor subfamily 5, group A, member 2
Hs.155485	HIP2	huntingtin interacting protein 2
Hs.75188	WEE1	WEE1 homolog (S. pombe)
Hs.9661	PSMB10	proteasome (prosome, macropain) subunit, beta type, 10
Hs.430146	AA488168	EST, Highly similar to dynactin 1, isoform 1; dynactin 1 (p150, Glued (Drosophila) homolog); p150, Glued (Drosophila) homolog; 150 kDa dynein-associated polypeptide; p150-glued [Homo sapiens] [H.sapiens]
Hs.171545	HRB	HIV-1 Rev binding protein
Hs.73947	PEPD	peptidase D
Hs.2316	SOX9	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)
Hs.211571	HCCS	holocytochrome c synthase (cytochrome c heme-lyase)
Hs.3352	HDAC2	histone deacetylase 2
Hs.132834	HEM1	hematopoietic protein 1
Hs.151518	TARBP1	TAR (HIV) RNA binding protein 1
Hs.75188	WEE1	WEE1 homolog (S. pombe)
Hs.239926	SC4MOL	sterol-C4-methyl oxidase-like
Hs.386741	ANXA7	annexin A7
Hs.170197	GOT2	glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2)
Hs.6335	MGC45562	hypothetical protein MGC45562
Hs.173936	IL10RB	interleukin 10 receptor, beta
Hs.75819	GPM6A	glycoprotein M6A
Hs.80680	MVP	major vault protein
Hs.155396	NFE2L2	nuclear factor (erythroid-derived 2)-like 2
Hs.80988	COL6A3	collagen, type VI, alpha 3
Hs.324473	MAPK1	mitogen-activated protein kinase 1
Hs.343586	ZFP36	zinc finger protein 36, C3H type, homolog (mouse)
Hs.95424	MAP4K1	mitogen-activated protein kinase kinase kinase kinase 1
Hs.37288	NR1D2	nuclear receptor subfamily 1, group D, member 2
Hs.170453	LOC158427	PP4189

Appendix 9

9.1 Table of genes with significantly higher polysome association in ZAP70 positive patients than ZAP70 negative patients.

Cluster Number	Gene Name	Gene Description
Hs.82535	SLC6A12	solute carrier family 6 (neurotransmitter transporter, betaine/GABA), member 12
Hs.154036	TSSC3	tumor suppressing subtransferable candidate 3
Hs.98243	SPINK2	serine protease inhibitor, Kazal type, 2 (acrosin-trypsin inhibitor)
Hs.348935	IGLL1	immunoglobulin lambda-like polypeptide 1
Hs.102950	IGLJ3	immunoglobulin lambda joining 3
Hs.348935	IGLL1	immunoglobulin lambda-like polypeptide 1
Hs.81454	KHK	ketohexokinase (fructokinase)
Hs.58927	NVL	nuclear VCP-like
Hs.117149	RGS9	regulator of G-protein signalling 9
Hs.79197	CD83	CD83 antigen (activated B lymphocytes, immunoglobulin superfamily)
Hs.40300	CAPN3	calpain 3, (p94)
Hs.2490	CASP1	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)
Hs.121509	COL11A2	collagen, type XI, alpha 2
Hs.83765	DHFR	dihydrofolate reductase
Hs.82283	MTR	5-methyltetrahydrofolate-homocysteine methyltransferase
Hs.727	INHBA	inhibin, beta A (activin A, activin AB alpha polypeptide)
Hs.350378	AA551124	Homo sapiens cDNA FLJ12825 fis, clone NT2RP2002800, mRNA sequence
Hs.172847	DNAJC4	DnaJ (Hsp40) homolog, subfamily C, member 4
Hs.58167	ZNF282	zinc finger protein 282
Hs.417144	H05619	EST, Moderately similar to NRTR_HUMAN Neurturin receptor alpha precursor (NTNR-alpha) (NRTNR-alpha) (TGF-beta related neurotrophic factor receptor 2) (GDNF receptor beta) (GDNFR-beta) (RET ligand 2) (GFR-alpha 2) [H.sapiens]
Hs.78473	NDST2	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 2
Hs.405998	AA256532	Human insulin-like growth factor 1 receptor mRNA, 3' sequence, mRNA sequence
Hs.321653	FLJ12770	hypothetical protein FLJ12770
Hs.184669	ZNF144	zinc finger protein 144 (Mel-18)
Hs.198515	DRIL1	dead ringer-like 1 (Drosophila)
Hs.109606	CORO1A	coronin, actin binding protein, 1A
Hs.351808	FGL2	fibrinogen-like 2
Hs.343874	PRODH	proline dehydrogenase (oxidase) 1
Hs.406050	DNALI1	dynein, axonemal, light intermediate polypeptide 1
Hs.130685	LTB4R2	leukotriene B4 receptor 2
Hs.104576	CHST1	carbohydrate (keratan sulfate Gal-6) sulfotransferase 1
Hs.92002	GNAZ	guanine nucleotide binding protein (G protein), alpha z polypeptide
Hs.44205	N50745	Unknown (protein for MGC:32686) [Homo sapiens], mRNA sequence
Hs.78146	PECAM1	platelet/endothelial cell adhesion molecule (CD31 antigen)
Hs.296847	SPG7	spastic paraplegia 7, paraplegin (pure and complicated autosomal recessive)
Hs.151051	MAPK10	mitogen-activated protein kinase 10
Hs.12152	APMCF1	APMCF1 protein
Hs.80420	CX3CL1	chemokine (C-X3-C motif) ligand 1
Hs.372587	PGPL	Pseudoautosomal GTP-binding protein-like

Hs.2864	EEA1	early endosome antigen 1, 162kD
Hs.733	EPB42	erythrocyte membrane protein band 4.2
Hs.9280	LIAS	lipoic acid synthetase
Hs.15110	ZNF211	zinc finger protein 211
Hs.81071	ECM1	extracellular matrix protein 1
Hs.150926	FPGT	fucose-1-phosphate guanylyltransferase
Hs.166733	LNPEP	leucyl/cystinyl aminopeptidase
Hs.11700	R60807	Similar to hypothetical protein FLJ21394 [Homo sapiens], mRNA sequence
Hs.352116	H2AFB	H2A histone family, member B
Hs.77515	ITPR3	inositol 1,4,5-triphosphate receptor, type 3
Hs.171909	U2AF1RS2	U2 small nuclear ribonucleoprotein auxiliary factor, small subunit 2
Hs.167017	GABBR1	gamma-aminobutyric acid (GABA) B receptor, 1
Hs.170808	GAD2	glutamate decarboxylase 2 (pancreatic islets and brain, 65kDa)
Hs.75133	TFAM	transcription factor A, mitochondrial
Hs.88974	CYBB	cytochrome b-245, beta polypeptide (chronic granulomatous disease)
Hs.82646	DNAJB1	DnaJ (Hsp40) homolog, subfmaily B, member 1
Hs.85951	XPOT	exportin, tRNA (nuclear export receptor for tRNAs)
Hs.26401	TNFSF12	tumor necrosis factor (ligand) superfamily, member 12
Hs.181107	ANXA13	annexin A13
Hs.77729	OLR1	oxidised low density lipoprotein (lectin-like) receptor 1
Hs.82401	CD69	CD69 antigen (p60, early T-cell activation antigen)
Hs.288856	PFDN5	prefoldin 5
Hs.75410	HSPA5	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)
Hs.82251	MYO1E	myosin IE
Hs.79440	KOC1	IGF-II mRNA-binding protein 3
Hs.119475	CIRBP	cold inducible RNA binding protein
Hs.355934	CARP	cardiac ankyrin repeat protein
Hs.195432	ALDH2	aldehyde dehydrogenase 2 family (mitochondrial)
Hs.84229	SFRS8	splicing factor, arginine/serine-rich 8 (suppressor-of-white-apricot homolog, Drosophila)
Hs.180930	BTAF1	BTAF1 RNA polymerase II, B-TFIID transcription factor-associated, 170kDa (Mot1 homolog, S. cerevisiae)
Hs.84673	TNNI1	troponin I, skeletal, slow
Hs.351863	TST	thiosulfate sulfurtransferase (rhodanese)
Hs.86122	GRCA	likely ortholog of mouse gene rich cluster, A gene
Hs.82027	OCA2	oculocutaneous albinism II (pink-eye dilution homolog, mouse)
Hs.386793	GPX3	glutathione peroxidase 3 (plasma)
Hs.182825	RPL35	ribosomal protein L35
Hs.37055	FGF5	fibroblast growth factor 5
Hs.26194	IAN4L1	immune associated nucleotide 4 like 1 (mouse)
Hs.8906	T71551	Homo sapiens clone 24889 mRNA sequence
Hs.406397	GFAP	glial fibrillary acidic protein
Hs.65424	TNA	tetranectin (plasminogen binding protein)
Hs.279032	HUMGT198A	GT198, complete ORF
Hs.179665	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
Hs.400353	DDIT3	DNA-damage-inducible transcript 3
Hs.1244	CD9	CD9 antigen (p24)
Hs.17466	RARRES3	retinoic acid receptor responder (tazarotene induced) 3
Hs.296327	RIPK1	receptor (TNFRSF)-interacting serine-threonine kinase 1
Hs.78781	VEGFB	vascular endothelial growth factor B
Hs.135626	CMA1	chymase 1, mast cell
Hs.179825	RANBP2L1	RAN binding protein 2-like 1
Hs.90303	TSC2	tuberous sclerosis 2
Hs.154207	CENPC1	centromere protein C 1
Hs.306220	CYP3A43	cytochrome P450, subfamily IIIA, polypeptide 43
Hs.349111	AA158003	Hypothetical protein [Homo sapiens], mRNA sequence

Hs.122116	RUNX2	runt-related transcription factor 2
Hs.103755	RIPK2	receptor-interacting serine-threonine kinase 2
Hs.110708	SGCE	sarcoglycan, epsilon
Hs.5038	NTE	neuropathy target esterase
Hs.1799	CD1D	CD1D antigen, d polypeptide
Hs.3828	MVD	mevalonate (diphospho) decarboxylase
Hs.10306	NKG7	natural killer cell group 7 sequence
Hs.26126	CLDN10	claudin 10
Hs.146688	PTGES	prostaglandin E synthase
Hs.16362	P2RY6	pyrimidinergic receptor P2Y, G-protein coupled, 6
Hs.31218	SCAMP1	secretory carrier membrane protein 1
Hs.396547	H72030	Homo sapiens full length insert cDNA clone YS16F09, mRNA sequence
Hs.161362	PIN1	protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting 1
Hs.154868	CAD	carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase
Hs.18676	SPRY2	sprouty homolog 2 (Drosophila)
Hs.181243	ATF4	activating transcription factor 4 (tax-responsive enhancer element B67)
Hs.155935	C3AR1	complement component 3a receptor 1
Hs.129055	ODF2	outer dense fiber of sperm tails 2
Hs.28081	EIF3S4	eukaryotic translation initiation factor 3, subunit 4 delta, 44kDa

Appendix 10

10.1 Table of genes with significantly higher polysome association in B-CLL patients with mutated I_gV_H genes compared to patients with unmutated I_gV_H genes.

Cluster Number	Gene Name	Gene Description
Hs.91747	PFN2	profilin 2
Hs.118825	MAP2K6	mitogen-activated protein kinase kinase 6
Hs.417750	H22563	EST
Hs.79516	BASP1	brain abundant, membrane attached signal protein 1
Hs.91143	JAG1	jagged 1 (Alagille syndrome)
Hs.184402	CAMK1	calcium/calmodulin-dependent protein kinase I
Hs.7644	H1F2	H1 histone family, member 2
Hs.86978	PREP	prolyl endopeptidase
Hs.278589	GTF2I	general transcription factor II, i
Hs.112058	SIVA	CD27-binding (Siva) protein
Hs.20084	RXRA	retinoid X receptor, alpha
Hs.74101	SYK	spleen tyrosine kinase
Hs.172471	KCNAB1	potassium voltage-gated channel, shaker-related subfamily, beta member 1
Hs.20137	BIKE	BMP-2 inducible kinase
Hs.430146	AA488168	EST, Highly similar to dynactin 1, isoform 1; dynactin 1 (p150, Glued (Drosophila) homolog); p150, Glued (Drosophila) homolog; 150 kDa dynein-associated polypeptide; p150-glued [Homo sapiens] [H.sapiens]
Hs.112058	SIVA	CD27-binding (Siva) protein
Hs.79069	AI571464	Homo sapiens mRNA; cDNA DKFZp434B142 (from clone DKFZp434B142), mRNA sequence
Hs.433410	MNAT1	menage a trois 1 (CAK assembly factor)
Hs.2795	LDHA	lactate dehydrogenase A
Hs.86386	MCL1	myeloid cell leukemia sequence 1 (BCL2-related)
Hs.17483	CD4	CD4 antigen (p55)
Hs.3059	COPB	coatomer protein complex, subunit beta
Hs.622	BRAF	v-raf murine sarcoma viral oncogene homolog B1
Hs.156346	TOP2A	topoisomerase (DNA) II alpha 170kDa
Hs.21486	STAT1	signal transducer and activator of transcription 1, 91kDa
Hs.272529	GPLD1	glycosylphosphatidylinositol specific phospholipase D1
Hs.75741	ABP1	amiloride binding protein 1 (amine oxidase (copper-containing))
Hs.83760	TNNI2	troponin I, skeletal, fast
Hs.172182	PABPC1	poly(A) binding protein, cytoplasmic 1
Hs.75257	SLBP	stem-loop (histone) binding protein
Hs.84981	XRCC5	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining; Ku autoantigen, 80kDa)
Hs.380096	ACTR3	ARP3 actin-related protein 3 homolog (yeast)
Hs.184326	CDC10	CDC10 cell division cycle 10 homolog (S. cerevisiae)
Hs.5344	AP1G1	adaptor-related protein complex 1, gamma 1 subunit
Hs.293007	NPEPPS	aminopeptidase puromycin sensitive
Hs.406365	SOS2	son of sevenless homolog 2 (Drosophila)
Hs.74034	CAV1	caveolin 1, caveolae protein, 22kDa
Hs.410488	R39356	EST, Weakly similar to hypothetical protein FLJ20489 [Homo sapiens] [H.sapiens]
Hs.75730	SRPR	signal recognition particle receptor ('docking protein')
Hs.75196	BAT8	HLA-B associated transcript 8
Hs.73947	PEPD	peptidase D

Hs.2055	UBE1	ubiquitin-activating enzyme E1 (A1S9T and BN75 temperature sensitivity complementing)
Hs.250616	IDH3A	isocitrate dehydrogenase 3 (NAD+) alpha
Hs.89591	KAL1	Kallmann syndrome 1 sequence

Appendix 11

11.1 Table of genes with significantly higher polysome association in B-CLL patients with unmutated I_gV_H genes, compared to patients with mutated I_gV_H genes.

Cluster Number	Gene Name	Gene Description
Hs.727	INHBA	inhibin, beta A (activin A, activin AB alpha polypeptide)
Hs.119007	RAB4A	RAB4A, member RAS oncogene family
Hs.3057	ZNF74	zinc finger protein 74 (Cos52)
Hs.73849	APOC3	apolipoprotein C-III
Hs.76893	BDH	3-hydroxybutyrate dehydrogenase (heart, mitochondrial)
Hs.102950	IGLJ3	immunoglobulin lambda joining 3
Hs.150917	CTNNA2	catenin (cadherin-associated protein), alpha 2
Hs.76293	TMSB10	thymosin, beta 10
Hs.166733	LNPEP	leucyl/cystinyl aminopeptidase
Hs.388927	AA491227	ESTs, Weakly similar to A56419 kappaE3' enhancer-binding protein NF-E1 - human [H.sapiens]
Hs.69360	KNSL6	kinesin-like 6 (mitotic centromere-associated kinesin)
Hs.262476	AMD1	S-adenosylmethionine decarboxylase 1
Hs.1915	FOLH1	folate hydrolase (prostate-specific membrane antigen) 1
Hs.171945	PLA2R1	phospholipase A2 receptor 1, 180kDa
Hs.370622	W90381	Homo sapiens clone R19540 SNURF-SNRPN mRNA, downstream untranslated exons, alternatively spliced, mRNA sequence
Hs.30954	PMVK	phosphomevalonate kinase
Hs.267445	MAPK8	mitogen-activated protein kinase 8
Hs.68583	MIPEP	mitochondrial intermediate peptidase
Hs.82587	PLD1	phospholipase D1, phophatidylcholine-specific
Hs.94925	DHODH	dihydroorotate dehydrogenase
Hs.178738	CYP3A4	cytochrome P450, subfamily IIIA (niphedipine oxidase), polypeptide 4
Hs.32935	BRF1	BRF1 homolog, subunit of RNA polymerase III transcription initiation factor IIIB (S. cerevisiae)
Hs.169756	C1S	complement component 1, s subcomponent
Hs.352341	STCH	stress 70 protein chaperone, microsome-associated, 60kDa
Hs.10712	PTEN	phosphatase and tensin homolog (mutated in multiple advanced cancers 1)
Hs.59545	TRIM38	tripartite motif-containing 38
Hs.21365	NAP1L3	nucleosome assembly protein 1-like 3
Hs.272429	CASR	calcium-sensing receptor (hypocalciuric hypercalcemia 1, severe neonatal hyperparathyroidism)
Hs.1501	SDC2	syndecan 2 (heparan sulfate proteoglycan 1, cell surface-associated, fibroglycan)
Hs.155356	MGC2840	hypothetical protein MGC2840 similar to a putative glucosyltransferase
Hs.315177	IFRD2	interferon-related developmental regulator 2
Hs.433619	PLK	polo-like kinase (Drosophila)
Hs.347508	BG185544	Homo sapiens cDNA: FLJ23482 fis, clone KAIA03142, mRNA sequence
Hs.77432	EGFR	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
Hs.367762	KRT6A	keratin 6A
Hs.102484	GSTA3	glutathione S-transferase A3
Hs.170027	MDM2	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)
Hs.184014	RPL31	ribosomal protein L31

Hs.89695	INSR	insulin receptor
Hs.75133	TFAM	transcription factor A, mitochondrial
Hs.17778	NRP2	neuropilin 2
Hs.121576	MYO1B	myosin IB
Hs.348935	IGLL1	immunoglobulin lambda-like polypeptide 1
Hs.350077	RPL21	ribosomal protein L21
Hs.323383	ALAS2	aminolevulinate, delta-, synthase 2 (sideroblastic/hypochromic anemia)
Hs.118442	CCNC	cyclin C
Hs.382777	AA644191	ESTs, Moderately similar to A54869 ADP-ribosylation factor-like 3 - human [H.sapiens]
Hs.72927	IL7	interleukin 7
Hs.42957	METTL1	methyltransferase-like 1
Hs.169825	COL4A5	collagen, type IV, alpha 5 (Alport syndrome)
Hs.78781	VEGFB	vascular endothelial growth factor B
Hs.102135	SSR4	signal sequence receptor, delta (translocon-associated protein delta)
Hs.172550	PTBP1	polypyrimidine tract binding protein 1
Hs.46700	ING1	inhibitor of growth family, member 1
Hs.387381	H15703	ESTs
Hs.25511	TGFB1I1	transforming growth factor beta 1 induced transcript 1
Hs.241493	AA281731	Similar to phorbol-12-myristate-13-acetate-induced protein 1 [Homo sapiens], mRNA sequence
Hs.181243	ATF4	activating transcription factor 4 (tax-responsive enhancer element B67)
Hs.9736	PSMD3	proteasome (prosome, macropain) 26S subunit, non-ATPase, 3
Hs.77221	CHK	choline kinase
Hs.110	KIAA0436	putative L-type neutral amino acid transporter
Hs.241570	TNF	tumor necrosis factor (TNF superfamily, member 2)
Hs.78765	ZNF32	zinc finger protein 32 (KOX 30)
Hs.113882	GABRD	gamma-aminobutyric acid (GABA) A receptor, delta
Hs.389933	RPS4X	ribosomal protein S4, X-linked
Hs.75217	MAP2K4	mitogen-activated protein kinase kinase 4
Hs.38069	C8B	complement component 8, beta polypeptide
Hs.28853	CDC7L1	CDC7 cell division cycle 7-like 1 (S. cerevisiae)
Hs.30941	CACNB2	calcium channel, voltage-dependent, beta 2 subunit
Hs.172847	DNAJC4	DnaJ (Hsp40) homolog, subfamily C, member 4
Hs.24763	RANBP1	RAN binding protein 1
Hs.323910	ERBB2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)
Hs.23205	MPP2	membrane protein, palmitoylated 2 (MAGUK p55 subfamily member 2)

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