MYC FAMILY OF GENES; STRUCTURE, EXPRESSION AND ACTIVATION IN TUMOR PATHOGENESIS

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STOCKHOLM 1989

Doctoral Dissertation 1989
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ABSTRACT

The aim of this study has been to identify the structural changes during c-*myc* activation in relation to tumor pathogenesis, to characterize the B*myc* gene, and to compare the structure and expression of *myc* gene family members.

The *myc* family members are dispersed over the rat and mouse genomes and are localized on different chromosomes. C-*myc* and N*myc* encode proteins that bind to different nuclear structures.

Aberrant expression of c-*myc* is believed to contribute to the pathogenesis of many different tumors. Our studies on the rat immunocytoma have shown that chromosome translocations juxtapose c-*myc* and IgH-derived sequences in three B cell tumors in three different species. Juxtaposition of c-*myc* to an immunoglobulin locus and subsequent constitutive expression is regarded as an essential step in the genesis of mouse plasmacytoma, rat immunocytoma, and human Burkitt lymphoma.

C-*myc* is regularly expressed in a wide variety of proliferating cells while N*myc* and L*myc* expression is limited to certain developmental stages and cell types. B*myc* has been isolated on the basis of the homology to c-*myc* second exon. Many tissues express B*myc*, independently of their developmental stage. During the embryonic development of the mouse and rat, c-*myc*, N*myc*, L*myc*, and B*myc* are expressed in different regions, and the genes show different expression pattern in F9 embryonal carcinoma cells during growth stimulation, suggesting independent regulation. C-*myc*, N*myc*, and L*myc* are downregulated during differentiation of F9 cells to visceral endoderm, while B*myc* is constantly expressed at low level in all stages. Inhibition of protein synthesis induces an elevation of c-*myc*, L*myc*, and B*myc* transcripts, suggesting that the genes are downregulated by a short-lived protein(s). Mitogenic stimulation with insulin and transferrin do not affect N*myc*, L*myc*, and B*myc* mRNA, while c-*myc* is upregulated.

The rat B*myc* gene contains sequences related to the central part of c-*myc*, namely the first intron and the second exon, and the noncoding part of the third exon. The homology drops in the 3´ part of the c-*myc* second exon, but continues in the noncoding part of the third exon. The total predicted coding region of the B*myc* has been sequenced and a bacterial tryptophanE fusion protein has been made. The lack of coding sequences in B*myc*, corresponding the third exon of the c-*myc*, which is thought to be important for nuclear localization, DNA binding, transforming activity, and the putative protein destabilation signal, suggests that the B*myc* and c-*myc* gene products may have distinct activities. Still, one of the two putative domains responsible for transformation activity of c-*myc* is found in the B*myc* sequence. These findings are discussed in the context of potential functional domains and the possibility of overlapping and distinct activities of *myc* -family proteins.

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ISBN 91-7900-820-8
Akademitryck, Edsbruk, 1989

This thesis is based on the following papers, which will be referred to by their Roman numerals:

I. **Ingvarsson, S.**, Asker, C., Wirschubsky, Z., Szpirer, J., Levan, G., Klein, G., and Sümegi, J. 1987. Mapping of L*myc* and N*myc* to rat chromosomes 5 and 6. *Som. Cell Molec. Genet.*, **13,** 335-339.

II. **Ingvarsson, S.**, Asker, C., Axelson, H., Klein, G., and Sümegi, J. 1988. Structure and expression of B*myc*, a new member of the *myc* gene family. *Mol. Cell. Biol.*, **8,** 3168-3174.

III. Asker, C., Steinitz, M., Andersson, K., Sümegi, J., Klein, G., and **Ingvarsson, S.** Nucleotide sequence of the rat B*myc* gene. Manuscript.

IV. Pear, W.S., **Ingvarsson, S.**, Steffen, D., Munke, M., Francke, U., Bazin, H., Klein, G., and Sümegi, J. 1986. Multiple chromosomal rearrangements in a spontaneously arising t(6:7) rat immunocytoma juxtapose c-*myc* and immunoglobulin heavy chain sequences. *Proc. Natl. Acad. Sci. USA*, **83,** 7376-7380.

V. **Ingvarsson, S.**, Wirschubsky, Z., Szpirer, J., Levan, G., Klein, G., and Sümegi, J. 1987. The rat *Mis/Pvt* -1 locus is syntenic with c-*myc* on chromosome 7. *Cytogenet. Cell Genet.*, **45,** 174-176.

VI. Henrikson, M., Classon, M., **Ingvarsson, S.**, Koskinen, P., Sümegi, J., Klein, G., and Thyberg, J. 1988. Elevated expression of c-*myc* and N-*myc* produces distinct changes in nuclear fine structure and chromatin organization. *Oncogene*, **3,** 587-593.

VII. Sejersen, T., Rahm, M., Szabo, G., **Ingvarsson, S.**, and Sümegi, J. 1987. Similarities and differences in the regulation of N-*myc* and c-*myc* genes in murine embryonal carcinoma cells. *Exp. Cell. Res.*, **172,** 304-317.

VIII. **Ingvarsson, S.**, Sundaresan, S., Jin, P., Francke, U., Asker, C., Sümegi, J. Klein, G., and Sejersen, T. 1988. Chromosome localization and expression pattern of L*myc* and B*myc* in murine embryonal carcinoma cells. *Oncogene,* **3,** 679-685.

ABBREVIATIONS

ActD	Actinomycin D
ANLL	Acute nonlymphatic leukemia
BL	Burkitt´s lymphoma
bp	basepair
CAT	Chloramphenicol acetyl transferase
CH	Cycloheximide
DM	Double minute
DMSO	Dimethyl sulfoxide
EBV	Epstein-Barr virus
EC	Embryonal Carcinoma
Eμ	Immunoglobulin enhancer
HSR	Homogeneously staining region
Ig	Immunoglobulin
IgH	Immunoglobulin heavy chain
Igk	Immunoglobulin kappa chain
Igl	Immunoglobulin lambda chain
INF	Interferon
Kb	Kilobasepairs
MEL	Murine erythroleukemia
MPC	Mouse plasmacytoma
NB	Neuroblastoma
RA	Retinoic acid
REF	Rat embryo fibroblast
RIC	Rat immunocytoma
SCLC	Small Cell Lung Cancer
TPA	12-O-tetradecanoulphorbol acetate
VitD	Vitamin D, dihydroxyvitamin D3

CONTENTS

1. INTRODUCTION.. 1
2. SCOPE OF THE THESIS.. 2
3. *MYC* FAMILY OF GENES.. 2
4. THE *MYC* GENES.. 4
   4A. Chromosome locations.. 4
   4B. Gene structure... 6
      4B1. c-*myc*... 6
      4B2. N*myc*.. 8
      4B3. L*myc*.. 8
      4B4. B*myc*.. 9
5. ACTIVATION OF *MYC*.. 13
   5A. Chromosome translocations..................................... 13
      5A1. Burkitt lymphoma.. 14
      5A2. Mouse plasmacytoma.. 16
      5A3. Rat immunocytoma... 17
      5A4. The *PVT* locus... 18
   5B. Gene Amplification.. 19
   5C. Viral transduction and viral insertions......................... 21
6. TRANSFORMATION BY *MYC*................................. 22
7. TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION OF *MYC* EXPRESSION.................. 23
8. CHARACTERIZATION OF *MYC* PROTEINS.......... 24
9. *MYC* EXPRESSION IN RELATION TO CELL PROLIFERATION AND DIFFERENTIATION 27
10. CONCLUSIONS.. 31
11. ACKNOWLEDGEMENTS... 32
12. REFERENCES.. 33

1. INTRODUCTION.

The retroviral oncogenes have their origins as cellular proto-oncogenes (Stehelin *et al.*, 1976), and characterization of a variety of these genes has led to the idea that they may be involved in regulation of normal cell proliferation and differentiation (for review, see Bishop,1983; Bishop, 1987). Structural and functional alterations of these proto-oncogenes caused by point mutations, chromosomal abberations, gene amplifications, or proviral insertions are strongly implicated in the genesis and progression of various tumors. Although oncogenes have one common denominator, namely participation in the regulation of cell divison, they fall into several unrelated categories; growth factors, growth factor receptors, protein kinases, signal transducers, nuclear oncogenes, and/or transcriptional factors. Some other genes can influence tumor development in a positive or negative direction (for review, see Klein, 1988). Oncogenes activated by regulatory or structural changes may favor tumor development while tumor suppressor gene can counteract it. A third group of genes can modulate secondary properties of the tumor, like invasiveness, metastatic ability or immunogenicity.

A set of oncogenes encoding nuclear proteins share some properties, including phosphorylation, rapid turnover, and DNA-binding activity of their products (Curran *et al.*, 1984; Hann *et al.*, 1984; Klempnauer *et al.*, 1984; Oren *et al.*, 1981; for a review see Eisenman and Thompson,1986; Müller *et al.*, 1988). Another characteristic of these genes is the ability to alter their expression in response to modulators for cell proliferation and differentiation (Curran and Morgan, 1985; Greenberg and Ziff, 1984; Hirai *et al.*, 1989; Kelly *et al.*, 1983; Mitchell *et al.*, 1985; Reich and Levine, 1984; Torelli *et al.*, 1985); *myc* and *fos* are prototypes of this group of genes. The biochemical function of the *myc* product is still not established although much is known indirectly about it. Experimental evidence has been obtained that products of *myc* and *fos* can stimulate transcription of other genes, suggesting that their normal function is regulation of transcription (Distel *et al.*, 1987; Kingston *et al.*, 1984; Setoyama *et al.*, 1986). C-*myc* has also been implicated to have a role in DNA synthesis (Classon *et al.*, 1987). Another nuclear oncogene, *jun*, has been shown to code for a transcriptional activatior protein, AP-1 (Bohman *et al.*, 1987), and is able to form protein complex together with *fos* (Chiu *et al.*, 1988; Sassone-Corsi *et al.*, 1988). These protein complexes seem to regulate gene expression and one of the target candidates for such regulation is the c-*myc* oncogene (Hay *et al.*, 1989). The *erb* A oncogene has been shown to be the thyroid hormone receptor

which is also a transcriptional activator (Sap *et al.*, 1986; Weinberger *et al.*, 1986).

2. SCOPE OF THE THESIS.

In our laboratory the work on the *myc* genes has been of special interest for the last years. These studies have mainly been on the activation of the c-*myc* gene by chromosomal translocation, gene amplification and viral insertion. The aim in this thesis is; 1) to study the activation of c-*myc* by chromosomal translocation in rat immunocytoma and 2) to characterize the B*myc* structure and expression in relation to other *myc* gene family members. The work on the B*myc* developed from work on the rat immunocytoma. The first B*myc* clone was detected in a rat genomic library, screened with a c-*myc* probe, in order to characterize the c-*myc* breakpoints in the rat immunocytoma. The comparative studies on B*myc* in relation to other members of the *myc* gene family have been on considered chromosome localization, nucleotide sequencing, and mRNA expression in developing rat tissues and in a differentiation inducible mouse emryonal carcinoma cell system.

3. *MYC* FAMILY OF GENES.

Families of genes consist of related genes that code for conserved amino acid domains that are thought to have similar functions within the cell. Such related genes are presumably derived from a common ancestral gene(s). Families of oncogenes are several and studies of tumor cells and mammalian DNA have identified a family of *myc* -related genes (Figure 1). The *myc* family members are dispersed over the genome and are localized on different chromosomes (paper I; paper II; paper VIII). The dispersed multigene *myc* family includes the c-*myc*, N*myc*, L*myc* (for review, see DePinho *et al.*, 1987), and B*myc* (paper II; paper III; paper VIII). C-*myc* and N*myc* encode proteins that bind to different nuclear structures (paper VI).

The *myc* gene was originally characterized as the transforming gene of a subclass of avian acute transforming retroviruses (Lautenberger *et al.*, 1981). The first cellular *myc* (c-*myc*) has been cloned and characterized on the basis of homology to v-*myc* (Vennström *et al.*, 1982). The N*myc* was originally isolated from neuroblastoma (Kohl *et al.*, 1983) and the L*myc* from small cell

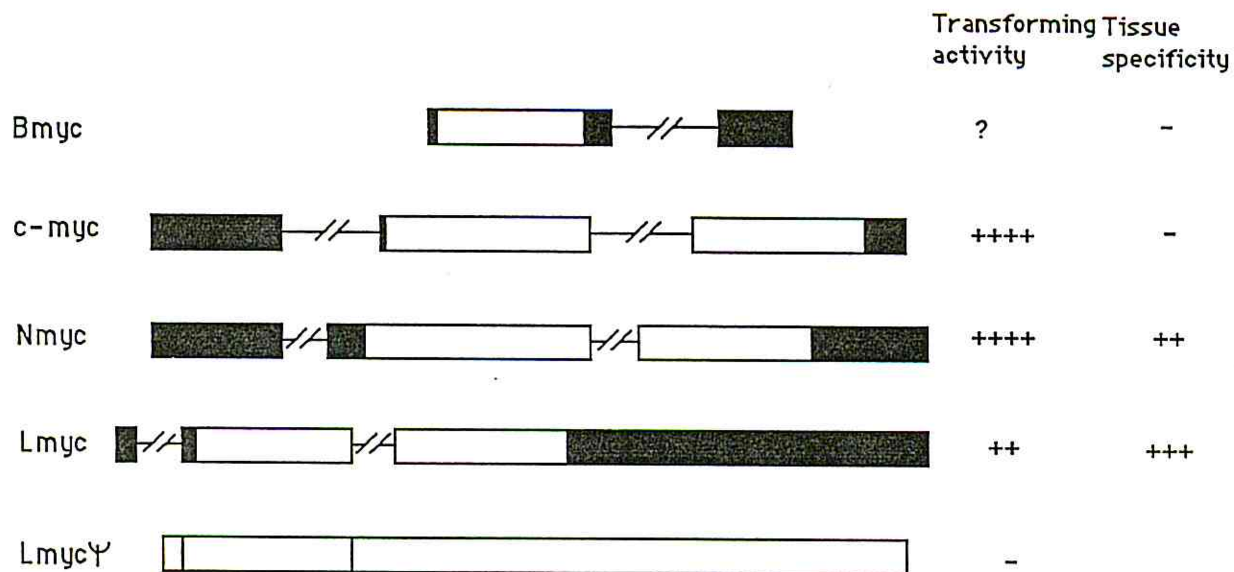

Figure 1. Structure, organization, and activity of the *myc* -family genes. Exons are indicated by boxes; open boxes represent translated regions, black boxes are untranslated regions, and lines represent intron or flanking sequences. (See text for details).

lung carcinoma (SCLC) (Nau *et al.*, 1985). N*myc* and L*myc* genes are amplified in the corresponding tumors and because of the homology to c-*myc*, probes from the c-*myc* gene could be used to detect the signals for the other *myc* genes. The B*myc* gene was isolated from normal liver library on the basis of c-*myc* homology. A less characterized *myc* gene is the R*myc*, which has been found to transform macrophages (Sklar *et al.*, 1985). Furthermore, there exists evidence for additional *myc* family members (Cole, 1986; Dalla-Favera *et al.*, 1982a; DePinho *et al.*, 1987a).

The c-*myc* is activated in many different tumors by various mechanisms; chromosome translocation (paper IV, for review, see Bornkamm *et al.*, 1988; Cory, 1986; Magrath, 1989), amplification (for review, see Alitalo, 1986; Collins and Groudine, 1988), viral insertion or viral transduction. The N*myc* gene is found to be activated by gene amplification in various tumors, like neuroblastoma, retinoblastoma and small cell lung cancer. The N*myc* gene has also been found to be activated in medulloblastomas, Wilm´s tumor and rhabdomyosarcoma by gene amplification (Mitani *et al.*, 1986; Norris *et al.*, 1988; Rouah *et al.*, 1989), and elevated expression in Wilms tumor has been reported without change in gene copy number (Nisen *et al.*, 1986; Shaw *et al.*, 1988). Furthermore, N*myc* has been found to be activated by retroviral insertion in *pim* -1 transgenic mice (VanLohuizen *et al.*, 1989a, b). L*myc* is

only found to be activated by gene amplification in small cell lung carcinoma. No correlation between B*myc* and tumor growth is documented so far.

The c-*myc* and B*myc* genes are expressed in many different tissues (Zimmerman *et al.*, 1986; paper II; paper VIII), at different levels, the c-*myc* expression being the highest in mesodermal and endodermal tissues (Schmid *et al.*, 1989). The main general difference is that c-*myc* is downregulated during lifespan while the B*myc* is expressed at a constant level. The N*myc* and L*myc* are much more restricted in their expression, mainly at fetal stages and in limited number of tissues (Mugrauer *et al.*, 1988; Semsei *et al.*, 1989; Zimmerman *et al.*, 1986). The expression of *myc* genes in different regions of developing rat tissues (paper II), and the differential expression pattern in F9 embryonic carcinoma cells during growth stimulation (paper VII; paper VIII), suggest independent regulation.

High expression of N*myc* in transgenic mice is associated with down-regulation of c-*myc* transcription (Dildrop *et al.*, 1989), suggesting cross-regulation between different members of the *myc* gene family. In line with these findings, c-*myc* expression is suppressed in fibroblasts transfected with high expressing v-*myc* or N*myc* constructs, or infected with a c-*myc* retrovirus (Cleveland *et al.*, 1988).

4. THE *MYC* GENES.

4A. Chromosome locations.

The chromosomal location of *myc* family genes and the *pvt* -1/*Mis* -1 locus has been determined in rat and mouse using somatic cell hybrids (Table 1). The chromosomal location of *myc* genes in mammalian species other than man has yielded additional clues about the involvement of the genes in certain types of neoplasia. For example, mouse plasmacytoma and rat immunocytoma involve transposition of c-*myc* to Ig loci (paper IV). That these gene rearrangements involved chromosome translocation was indicated when c-*myc* was assigned to mouse 15 (Dalla-Favera *et al.*, 1982c) and rat 7 (Sümegi *et al.*, 1983), the sites of translocation. The rat -mouse hybrid panel has been used to map the Ig heavy chain, kappa and lambda cluster to chromosomes 6, 4, and 11, respectively (Pear *et al.*, 1986b; Perlmann *et al.*, 1985; Wahlström *et al.*, 1988).

The finding that all 16 analysed RICs contain an identical t(6;7) translocation (Pear *et al.*, 1988a; Wiener *et al.*, 1982) is in contrast to approximately 20% of

Table 1. Chromosome locations•)

	Rat	Mouse	Human
c-*myc*	7	15D2D3	8q24
Pvt-1/*Mis*-1	7	15D2D3	8q24
N*myc*	6	12	2p23-24
L*myc*	5	4	1p32
B*myc*	3	2	

•) References; Paper I; paper II; paper V; paper VIII; Dalla-Favera *et al.*, 1982c; Campell *et al.*, 1989; Nau *et al.*, 1985; Schwab *et al.*, 1984; Sümegi *et al.*, 1983).

BLs and 10% of MPCs in which the translocation involves the chromosomes containing the c-*myc* and one of the Ig light chain loci (Cory *et al.*, 1986). Our inability to detect variant translocations in the RIC may be a consequence of the biology of this tumor; or alternatively, our sample size of 16 tumors may be to small to detect an infrequent event. This led to the speculation that c-*myc* had dissociated from the *pvt* -1/*Mis* -1 locus in the rat; however the chromosome mapping of both genes shows that they are syntenic (paper V).

While the correlation between chromosome abnormalities and tumor growth in the rat, other than immunocytoma, is poorly characterized, several such correlations have been documented for the mouse. For example, partial deletion of region C and D on the B*myc* carrying mouse chromosome 2 was detected in 49 out of 52 irradiation-induced myeloid leukemias (Hayata *et al.*, 1983). Out of these 52 myeloid leukemias, 6 cases of trisomy 6 and 4 cases of trisomy 15 occurred in addition to the deletion. It was thus concluded that trisomies 6 and 15 were secondary changes in myeloid leukemogenesis, while deletion 2C-D was the crucial event (Hayata *et al.*, 1983).

Comparative mapping of *myc* genes in different species has been of interest since they appear to be evolutionary very old. By mapping of *myc* genes in species other that man, additional information has been gathered concerning the evolution of gene families and gene linkage groups (paper I; paper II; paper VIII).

4B. Gene structure.

4B1. C-*myc* .

The c-*myc* is highly conserved in evolution and has been cloned from several vertebrate species from trout (VanBeneden *et al.*, 1986) to mammals. Limited homology has been found to the achaete-scute gene in *Drosophila melanogaster* (Villares *et al.*, 1987). Furthermore, *Drosophila* genomic and RNA sequences that hybridize with v-*myc* have been reported (Madhavan *et al.*, 1985; Shilo and Weinberg, 1981).

The c-*myc* has three exons, the first exon being mainly noncoding. There is also a large noncoding region in the 3′ part of the third exon (Figure 1; Figure 5). The first exon is the site of regulation, two promoters (P_1 and P_2) yield two differently sized mRNA (2.2 and 2.4 kb), and a block of transcription is located there (Eick and Bornkamm, 1986; Kerppola and Kane, 1988; Wright and Bishop, 1989). It has been shown that loss of the first exon increases the life time of the mRNA several fold (Eick *et al.*, 1985; Piechaczyk *et al.*, 1985; Rabbits *et al.*, 1985). It has been proposed that this post-transcriptional regulation is mediated by a hairpin structure between c-*myc* exon 1 and exon 2, that yields a recognition site for a double stranded specific RNAse (Gariglio *et al.*, 1987). Results from an *in vitro* system where the c-*myc* first exon was fused to mRNA of other genes suggested that the first exon is not sufficient to tag mRNA for rapid degradation (Pei and Calame, 1988). A truncation of the c-*myc* first exon results in loss of the larger protein (p67) (Hann *et al.*, 1988), that initiates from a CUG codon in the 3′ part of the first exon. The conservation of the c-*myc* first exon, as well as the noncoding part of the 3rd exon (Bernard *et al.*, 1983; Hayashi *et al.*, 1987), confirms the importance of these sequences. The human c-*myc* first exon has a putative open reading frame (Ferré *et al.*, 1987; Gazin *et al.*, 1986) but the function of such a protein remains unclear. This open reading frame does not exist in the mouse c-*myc*, still antibodies agains this putative peptide detects a specific signal, suggesting that a similar protein is coded from a different locus in mice (Dedieau *et al.*, 1988).

The noncoding part of the third exon has been shown to be important for the stability of the mRNA (Bonnieu *et al.*, 1988; Brewer and Ross, 1988; Jones and Cole, 1987). The breakpoint in T-cell leukemia in the destabilization motiv activates the c-*myc* (Finger *et al.*, 1988), shown by enhanced mRNA levels and tumorigenicity, and is the only documented case where *myc* can be tumorigenic under the control of its own promoter (Aghib, personal communication). The

poly(A) is also important in this aspect; it has been shown that poly(A)- c-*myc* mRNA is more stable that poly(A)+ in growing cells, while both forms are degraded rapidly when cells are induced to differentiate (Swartwout and Kimiburgh, 1989).

The c-*myc* gene product has been studied by systematic mutational analysis and how the mutation affect transforming activity, nuclear localization, DNA binding, and protein oligomerization (Dang and Lee, 1988; Dang *et al.*, 1989; Sarid *et al.*, 1987; Stone *et al* 1987). The c-*myc* gene can be divided into four regions according to functional analysis of mutated c-*myc* proteins (Figure 2).

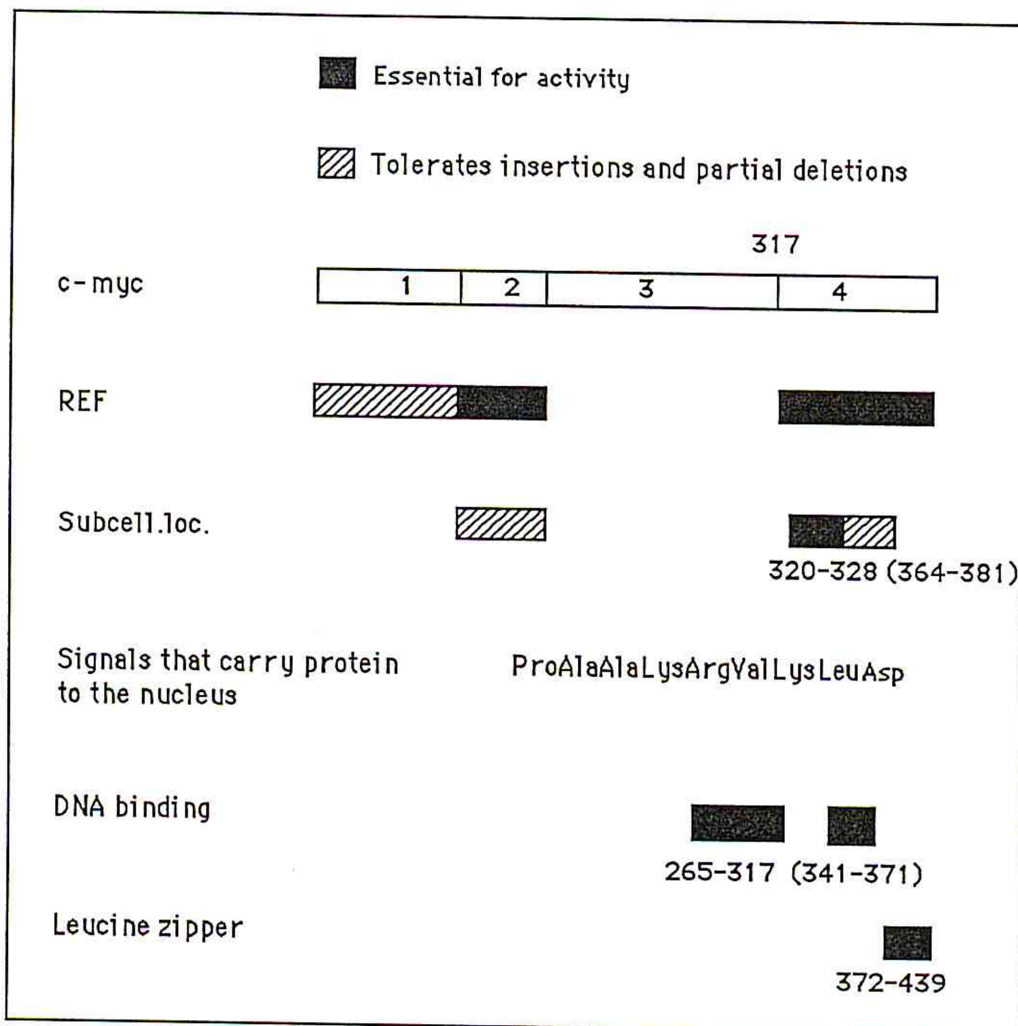

Figure 2. Functional regions of the c-*myc* protein. Regions 1 through 4 are defined in the text (Dang *et al.*, 1989; Dang and Bishop, 1988; Stone *et al.*, 1987).

Two regions important for the transforming activity of the c-*myc* gene have been localized in the second and the third exon, and the region important for DNA binding is located in the third exon. The putative nuclear migration signal has been localized in the third exon at amino acid 320 to amino acid 328 (Dang

and Lee, 1988). The "leucin Zipper" sequence located in the third exon of c-*myc*, responsible for oligomerization of the c-*myc* protein has been characterised by the same deletion mutants (Dang *et al.*, 1989). This DNA binding and dimerization motif is also found in N*myc* and L*myc* proteins, as well as in *MyoD* and drosophila proteins (Murre *et al.*, 1989). A putative protein destabilization signal (PEST sequence) is located on the second exon-third exon boundary (Rechsteiner *et al.*, 1987).

4B2. N*myc*.

The N*myc* gene has similar overall gene structure as c-*myc* with three exons and the first exon noncoding (DePinho *et al.*, 1986; Kohl *et al.*, 1986; Stanton *et al.*, 1986; Taya *et al.*, 1986). Certain regions of the coding sequence have high homology to c-*myc* ; seven such *myc* boxes are evident (DePinho *et al.*, 1986; Taya *et al.*, 1986). Five of these boxes are also found in the L*myc* gene (Legouy *et al.*, 1987). Like for c-*myc*, two proteins have been described from the N*myc* locus (Mäkäle *et al.*, 1989), but both initiate from a AUG codon in the 5´ region of exon 2, in contrast to the initiation of c-*myc* larger protein from the CUG codon in the first exon (Hann *et al.*, 1988). There is no homology between the N*myc* and c-*myc* in the first exon and third exon noncoding sequences, and the N*myc* third exon noncoding part is even larger than found in the c-*myc* (Kohl *et al.*, 1986; Stanton *et al.*, 1986). Alternative splicing of N*myc* first exon has been described (Stanton, personal communication), suggesting different transcripts with separate regulation. The N*myc* third exon noncoding region has been found to be a site of retroviral insertion (VanLohuizen *et al.*, 1989a, b).

4B3. L*myc*.

The L*myc* gene also has a three exon structure like the c-*myc* and N*myc*, but alternative splicing and polyA utilization can yield different mRNA forms. This complex mRNA processing results in gene structure consisting of two exons, either by not splicing out the first intron, or by utilization of alternative polyA, and truncation of the third exon sequences. As a result four to six different mRNA are detected in Northern blots, using probes from different regions of the L*myc* gene (Kaye *et al.*, 1988). The mRNA with truncated third exon sequence has been shown to produce cytosolic protein (DeGreve *et al.*, 1988; Ikegaki *et al.*, 1989). This is the first evidence that a *myc* family gene can code

for a cytosolic protein, otherwise they are considered to code exclusively for nuclear proteins. There is no evidence for cytosolic proteins from the c-*myc* or N*myc* locus, but interestingly the B*myc* has very high homology to the coding region of the c-*myc* second exon and lacks the third exon coding region (paper III), which have been shown to be important for the subcellular localization of the c-*myc* protein (Dang and Lee, 1988). It is not determined where the B*myc* protein is localized within the cell. Thus, certain similarities can be seen in the comparison of different L*myc* mRNA species to c-*myc* /B*myc* structure homology.

The L*myc* open reading frame is slightly smaller than for c-*myc* and N*myc* and has several high homology regions to both genes (Legouy *et al.*, 1987). Seven such homology boxes are found in comparison to N*myc*, and five of them are even highly conserved in the c-*myc*.

The third exon noncoding region of L*myc* is larger than for the c-*myc* and N*myc*, and is conserved in evolution (DePinho *et al.*, 1987b). The noncoding first exon is also conserved between species and contains a site for elongation termination (Krystal *et al.*, 1988) similar to c-*myc*. No such control of transcription has been shown for the N*myc* or the B*myc*. The L*myc* has a non-functional homolog, in the form of intronless pseudogene, on the human X chromosome (DePinho *et al.*, 1987b).

4B4. B*myc*.

We have isolated a genomic B*myc* clone from a rat genomic library on the basis of c-*myc* second exon homology (paper II). Furthermore we have isolated several cDNA clones from rat brain and kidney libraries, of major sizes 1.3 and 2.0 kb. (paper II, Figure 3). The 1.3 kb cDNA clone has an open reading frame of 178 amino acids, with homology to the first 3 *myc* boxes in addition to high homology to c-*myc* in the largest part of the remaining second exon coding sequence (paper III). The putative B*myc* product is only 41% the size of the c-*myc* protein. B*myc* is the first *myc* family member reported to lack completely coding sequence from the third exon, but L*myc* can code for such a protein by using alternative mRNA processing (Ikegaki *et al.*, 1989).
The 1.3 kb cDNA clone has been introduced into a bacterial tryptophan E protein expression vector and into SV40 expression vectors. In addition to the 308 amino acids from the bacterial trpE protein the pATH3-B*myc* encodes the entire putative B*myc* protein (178 amino acids) under the transcriptional and translational control signals provided by the vector. The expression of a B*myc*

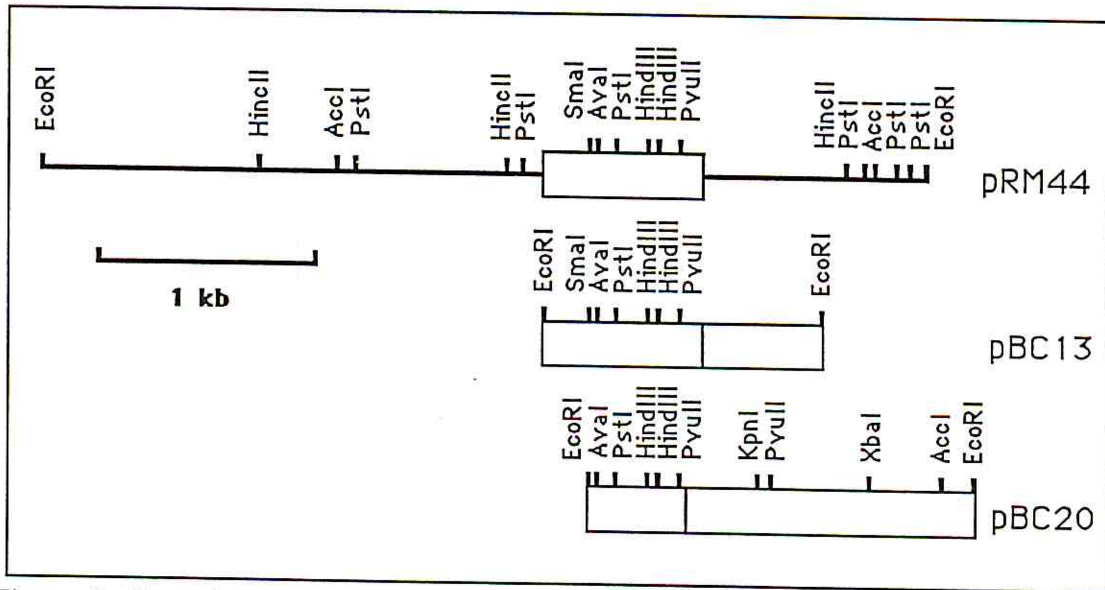

Figure 3. Organization of the B*myc* gene. Partial restriction map of the 4.4 kb genomic (pRM44), 1.3 kb cDNA (pBC13), and 2.0 cDNA (pBC20) rat B*myc* clones. One of the *Eco* RI sites in the genomic clone and both sites in the cDNA clones were created by linker addition during the cloning of the DNA. The boxes indicate the positions of putative exons.

protein, presumably from its own initiation codon, from the SV40 constructs, yields a 24 kd protein (Steinitz *et al.*, unpublished results).

The high homology between c-*myc* and B*myc* suggests that these conserved sequences may play similar general roles in the B*myc* and c-*myc* proteins. Still, the lack of the c-*myc* third exon coding sequence, thought to be important for nuclear localization, DNA binding, transforming activity, and protein oligomerization (Dang *et al.*, 1988; Dang *et al.*, 1989; Landschulz *et al.*, 1988; Sarid *et al.*, 1987; Stone *et al.*, 1987), suggests that the B*myc* and c-*myc* gene products have distinct activities. Of interest is that alternative processing of mRNA of another member of the *myc* gene family, the L*myc*, seems to create cytosolic L*myc* protein which lacks the 3rd exon coding sequence, in addition to the full length nuclear protein (DeGreve *et al.*, 1988; Ikegaki *et al.*, 1989; Kaye *et al.*, 1988). The putative protein destabilization signal, the PEST motiv present in c-*myc* (Rechsteiner *et al.*, 1988), is absent in the B*myc* gene.

Regions with sequence homology in several oncoproteins, where the predicted secondary structure consists of β-sheet and α-helix configuration, localize in domains of the respective proteins known to be required for transforming activity (Figge *et al.*, 1988). The human c-*myc* has two such regions at amino acid positions 342-354 and 117-126 (Figure 4). The region at amino acids 117-126 is considered to be less specific. Both of the regions identified in c-*myc* fall whithin larger regions which have been shown to be important for its transforming function (Sarid *et al.*, 1987; Stone *et al.*, 1987).

A

Protein	Species or Serotype	C (#)	.P.¤		D D#E
			ß-Turn		α-Helix
T-Ag	SV40	105 CSEE- -	MPSS	-	DDEATADS
E1A	Ad2,Ad5	124 CHEAGF	PPSD	-	DEDEEGEEFVLD
	Ad7	117 CYEEGF	PPSD	-	DEDGETEQS
	Ad12	109 CYEMG-	FPCS	-	DSEDEQDENGMAHVSASAAAAAADREREEFQLD
c-*myc*	Viral	325 CD- - - -	SPRT	L	DSEEND
	Mouse/Rat	342 CS- - - -	SPRS	S	DTEEND
	Human	342 CT- - - -	SPRS	S	DTEENV
	Chicken	319 CS- - - -	SPRT	S	DSEEND

B

Protein	Species	C (#)	.P.¤		E D#
			ß-Turn		α-Helix
c-*myc*	Viral	109 C- - - - -	DPDD	-	ESFVKSIIIQ
	Mouse/rat	118 C- - - - -	DPDD	-	ETFIK
	Human	117 C- - - - -	DPDD	-	ETFIK
	Chicken	103 C- - - - -	DPDD	-	ESFVKSIIIQ
B*myc*	Rat	106 C- - - - -	DPDD	-	ETFVK

Figure 4. Alignement of potential transforming regions of nuclear oncoproteins (modified from Figge *et al.*, 1988). (A) Alignements with descriptor 1. The symbol ¤ represents a polar amino acid without a positive charge: Ser, Thr, Asp., Glu, Asn, or Gln. The symbol # represents an amino acid with a negative or potential negative (via phosphorylation) charge: Asp, Glu, Ser, or Thr. Descriptor 1 contains five elements: Cys; sero to five amino acids of any type with the requirement that a residue from the # set must occur 1 or 2 positions downstream of the Cys; a predicted β-turn with a Pro in the second position and a residue from the ¤ set in the fourth position; zero or one amino acid of any type; and an α-helix with the tripeptide Asp-(Asp, Glu, Ser, or Thr)-Asp or Glu) within -5 to +7 residues of the predicted N terminus. (B) Additional alignements with descriptor 2. Descriptor 2 is identical to descriptor 1 except that the first element of the α-helix tripeptide can be Glu or Asp and the third element is omitted.

Amino acids 342-354 have homology to the transforming region of simian virus 40 large T and adenovirus E1A, while amino acids 117-126 have homology to B*myc*. Studies on c-*myc* and N*myc* hybrid constructs suggest that both the second and third exon of the c-*myc* are required to yield a product that shows

characteristic subnuclear localization, while in the case of the N*myc* the second exon is sufficient to give rise to a product that morphologically behaved like the N*myc* protein (Paper VI).

A short B*myc* sequence homologous to the noncoding c-*myc* sequence in the third exon seems to be coding. The homology to the c-*myc* third exon noncoding sequence continues in the intron sequence of B*myc* .

The two B*myc* cDNA clones isolated share the same sequence in the 5´ coding region but they have different 3´ exons (Figure 3). The 2.0 kb clone (pBC20) does not have the AUG initiation codon found in the 1.3 kb clone (pBC13), presumably due to improper transcription by reverse transcriptase in the library construction, but contains the termination codon at the same position. The 3´ region of the larger form of cDNA has no homology to the smaller form, suggesting alternative mRNA processing, yielding a transcript with distinct noncodon exons at the 3´ end. Several dissimilarities are found between these two cDNAs, e.g. the existence of additional polyA sequence and mRNA destabilization signals. The 3´ exon in the 2.0 kb cDNA clone is probably downstream of the 3´ exon in the 1.3 kb cDNA clone, as detected by genomic Southern blots of rat and mouse DNA using probes from several regions of the two different cDNA clones (unpublished observation).

In addition to the high homology of B*myc* to the 5´ part in the second exon of c-*myc* the B*myc* gene has homology to the first intron of c-*myc* (Paper III). This corresponds to the c-*myc* first intron homology between different species, suggesting that these DNA regions may be of functional importance. The homology drops in the 3´ part of the c-*myc* second exon but continues in the noncoding part of the third exon. There exists no direct evidence for promoter localization within the sequenced part of the B*myc* gene, but several possible TATA motivs are found in the 5´ sequence.

The upstream polyA addition, if such occurs, removes one of the destabilization sequences from the c-*myc* mRNA. This could also occur in the B*myc*, since the poly A addition is upstream of the destabilization sequences in the 3´ exon. Such a shortened c-*myc* mRNA may have a longer life and, therefore, may exist even in the absence of transcriptional activator. Although such shortened mRNA species has not been described, the conservation of the polyA addition signals (Bernard *et al.*, 1983; Hayashi *et al.*, 1987) hints at such regulatory mechanism beeing required at some stage in the life cycle.

5. ACTIVATION OF *MYC*.

C-*myc* is activated during oncogenesis by a variety of mechanisms and appears to play a central role in the etiology of many tumors. The abnormal, deregulated c-*myc* expression is a common feature of numerous malignancies and occurs by a variety of molecular mechanisms, which probably reflect the existence of multiple factors responsible for its normal control. Structural alterations of the c-*myc* locus have been most thoroughly investigated in mammalian B and T lymphoid neoplasias (for review, see Cory 1986). The various mechanisms of *myc* activation in many different tumors are; chromosome translocation (paper IV; for review, see Bornkamm *et al.*, 1988; Cory, 1986; Magrath, 1989), amplification (for review, see Alitalo and Schwab, 1986; Collins and Groudine, 1988), viral insertion or viral transduction (for review, see Clurman and Hayward, 1988; Nusse and Berns, 1988) (Table 2, Figure 5).

Table 2. Different mechanisms of *myc* activation:

	Amplification	Viral insertion	Translocation
c-*myc*	+	+	+
N*myc*	+	+	?
L*myc*	+	?	?
B*myc*	?	?	?

5A. Chromosome translocations.

Studies of the c-*myc* locus provided the first evidence that cellular oncogene activation was linked to the consistent chromosomal translocations associated with certain tumors. The chromosome translocations characteristic of certain B lymphoid tumors associate the c-*myc* oncogene and immunoglobulin (Ig) loci. This juxtaposition of c-*myc* to an Ig gene is the clearest example of tissue-specific oncogene activation by a purely cellular mechanism. The molecular anatomy of the translocation is closely similar in humans, mice, and rats.

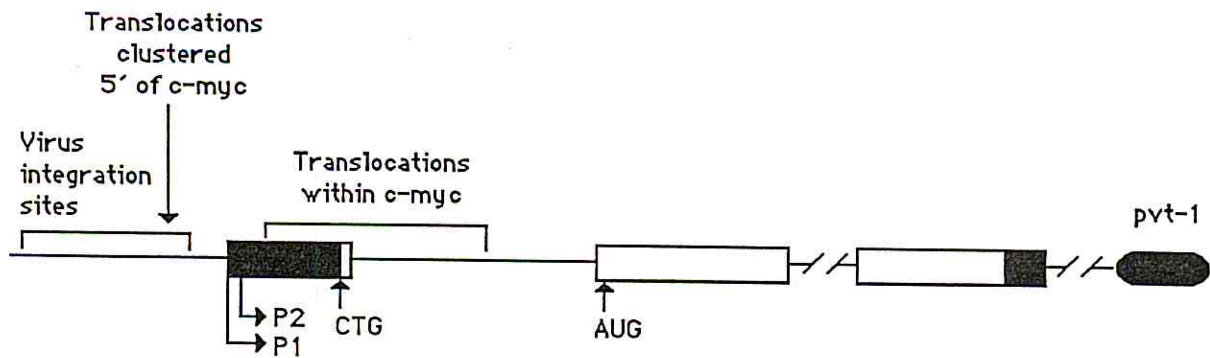

Figure 5. Topology of the c-*myc* locus. The first of three *myc* exons is mainly non-coding and the open reading fram of the c-*myc* polypeptide begins in the 3′ end of this exon. The locations of the gene′s normal promoters (P_1 and P_2) are indicated. The sites of chromosome translocation clustered upstream and within the gene are shown above the map. The predominant insertion region of MuLV and MCF proviruses in murine T lymphomas is 0.7-2.0 kb 5′ of the gene′s first exon. *Pvt* -1 is a locus distant from c-*myc* where variant translocations and proviral insertions have been observed in murine plasma cell tumors and T lymphomas. In BL and RIC some translocations have also been reported at greater distances 5′ of *myc* .

5A1. Burkitt Lymphoma (BL).

Burkitt lymphoma (BL) is an agressive B-cell neoplasm that occurs mainly in children and represents one of the most striking examples of a correspondence between reciprocal translocation, activation of c-*myc*, and neoplasia. BL may include several forms. Endemic Burkitt lymphoma (eBL) is the most characteristic form, which occurs in regions where there is a high incidence of malaria. The eBL manifests a dramatic tumor formation with a common localization in the jaws and has a peak incidence in 5-6 years olds. eBL has a very high and consistent association with Epstein-Barr virus (EBV) infection, most of the tumors carry the EBV-genome .

Sporadic Burkitt lymphoma (sBL), in contrast, rarely involves the jaws, tumors are EBV negative, and occurs principally in the USA and Europe. The eBL is confined to solid tumors while sBL is usually more dissociated and generalized. Both forms of tumor cells contain invariably specific reciprocal chromosomal translocation which involve the Ig loci on chromosome 2, 14, or 22 and the c-*myc* oncogene locus on chromosome 8 (for review, see Klein 1985). It is believed that these translocation deregulate the *myc* gene by bringing it into or near Ig chromosomal domains. A number of distinct and attractive models have been proposed for the mechanism of c-*myc* activation that results from these translocation; inactivation of putative 5′ regulatory

sequences by mutation (Remmers *et al.*, 1986; Wiman *et al.*, 1984), transriptional activation by known Ig enhancer element (Hayday *et al.*, 1984) or by an as yet unidentified Ig enhancer capable of exerting transcriptional influence over distances of up to 100 kilobases (kb). In most cases of BL with t(8;14) translocation the IgH enhancer is on the reciprocal fragment of the translocation, unavailable for the c-*myc* gene. This suggests that the c-*myc* gene activation is an indirect rather that a direct effect of the Ig enhancer. This assumption is based of the observation that Ig genes remain active *in vivo* even if the enhancer was lost by deletion (Klein *et al.*, 1984; Wabl and Burrows, 1984). Once the Ig locus is transcriptionally active, removal of the enhancer should have no major effect on transcription.

Difference is found in the molecular architecture of eBL and sBL. There are differences in the chromsosme 8 breaksites in the two forms, in sBL most of the breaks occur in exon 1 and intron 1 of c-*myc* while in the case of eBL the breakpoints are further upstram of the gene (Pelicci *et al.*, 1986). The translocation breakpoints on chromosome 14 are associated with V-D-J regions in endemic BL cells and with switch regions in sporadic BL cells (Haluska *et al.*, 1986). The c-*myc* locus of endemic BL are not rearranged but accumulate mutations in their 5´ flanking and first exon sequences (Pelicci *et al.*, 1986). Based on sequence homology between c-*myc* first exon sequence and J region of the Ig locus, and the mutations in the same region in endemic BL, it has been suggested that the mechanism behind the translocation event is enzyme recognition mistakes (Morse *et al.*, 1989). This activation mechanism may reflect the less differentiated characteristics of eBL cells.

The nucleotide sequence in the first exon of c-*myc* is highly conserved in evolution (Bernard *et al.*, 1983; Hayashi *et al.*, 1987), suggesting a regulatory role. In the cases of sBL where the 1st exon is broken or lost, several deregulations of the c-*myc* gene have been documented. A block of elongation has been demonstrated within the 1st exon of c-*myc* that is lost in several BL (Cesarman *et al.*, 1987). DMSO induces a block of c-*myc* RNA elongation in differentiating HL60, and in the human B cell line BJAB (Bentley and Groudine, 1986a). In BL cell lines with variant translocations, which are characterized by mutations in and around the first c-*myc* exon, DMSO is not capable of inducing the RNA elongation block (Eick *et al.*, 1988).

The larger form of the c-*myc* protein (p67) initiates in the first exon and several BL have lost this product (Hann *et al.*, 1988). The first exon of the c-*myc* has also been suggested to be important for mRNA stability, as shown by longer half life of the messenger in BL and mouse plasmacytoma (MPC) with truncated

c-*myc* gene compared to control cell lines (Eick *et al.*, 1985; Piechaczyk *et al.*, 1985; Rabbits *et al.*, 1985).

Important upstream elements that normally regulate the expression of c-*myc* are not only located in exon 1 but also in its 5´ region. A cis-acting negative regulatory elements with the opposite properties of a transcriptional enhancer (denoted the "*myc* dehancer") has been localized to a 716 bp DNA segment 5´ of the murine c-*myc* first exon (Remmers *et al.*, 1987; Weisinger *et al.*, 1988) and similar negative elements have been identified upstream of the human c-*myc* gene (Chung *et al.*, 1986; Hay *et al.*, 1987; Hay *et al.*, 1989; Lipp *et al.*, 1987). The nuclear factor binding sites within the "dehancers" negative elements are conserved to varying degrees in mice and humans (Weisinger *et al.*, 1988). It is speculated that loss of such a regulatory region by chromosome translocation or other rearrangements, would result in higher c-*myc* expression.

Another indicator of perturbated c-*myc* transcription in BL, MPC and RIC cells is the shift in promoter usage in cell lines with a nontruncated c-*myc* in favor of P1 (Axelson *et al.*; Taub *et al.*, 1984; Yang *et al.*, 1985).

5A2. Mouse plasmacytoma (MPC).

As a confirmation that the chromosomal translocation associated with BL is of pathogenetic significance is the observation that homologous translocation which result in the juxtaposition of c-*myc* and immunoglobulin sequences occur in mouse plasmacytoma (MPC) and rat immunocytoma (RIC) (Adams *et al.*, 1983; Marcu *et al.*, 1983; paper IV). The MPC contains translocations, similar to BL, between the *myc* carrying chromosome 15 and one of the Ig loci on chromsome 6, 12, or 16 (Ohno *et al.*, 1979; Sugiyama *et al.*, 1989; for review, see Cory, 1986; Mushinski, 1988). By chromosome sorting techniques we have shown that the c-*myc* oncogene is translocated, together with the *pvt* -1 loci, from chromosome 15 to chromosome 12 (Wirschubsky *et al.*, 1985), in line with results obtained by using somatic cell hybrids (Erikson *et al.*, 1985).

The development of transgenic mice have given additional information on the importance of *myc* activation in the development of pre-B and B cell lymphomas (Adams *et al.*, 1985; for review see Adams, 1988). The resulting tumors that arose in cell lineages with a high level of the transgene expression were oligo or monoclonal, showing that at least one additonal change was required. Furthermore, the importance of the Eμ in the transgenic mice system strongly suggests that a positive control input from the Ig loci is necessary in the

MPC formation and that truncation of the *myc* locus is probably not enough to activate the c-*myc*. The failure of first exon mutant Eμ-*myc* constructs to cause tumors in transgenic mice also points towards the importance of the Ig transposition (Adams, personal communication). Thus, as a general conclusion, loss or mutation of the normal regulatory region in the 5′ part of the c-*myc* gene is not sufficient to cause gene activation and a tumor phenotype; a positive input from one of the Ig loci is needed. The substitution of N*myc* into the Eμ constructs is additional evidence of the *myc* role in pre-B and B-cell lymphoid malignancies (Dildrop *et al.*, 1989; Rosenbaum *et al.*, 1989).

In transgenic mice the c-*myc* expression is not exceedingly high in the premalignant stage as well as in B-cell tumors (Alexander *et al.*, 1987). This suggests that the failure to downregulate c-*myc* is critical, rather that the absolute level of expression. Similarly, the amount of c-*myc* mRNA in BL, MPC and RIC cells is quite variable and does not exceed that in lymphocytes after induction of proliferation with mitogens (Keath *et al.*, 1984b, Pear *et al.*, 1988a).

As a further confirmation of the role of *myc* deregulation in MPC it has been shown that chromosome translocation can be replaced by retroviruses that express v-*myc* (Potter *et al.*, 1987). Infection of pristane-treated Balb/c mice with a retrovirally activated *myc* construct (J3) has dramatically reduced the latency period of plasmacytoma development. Tumors that expressed the v-*myc* insert had no translocations (Potter *et al.*, 1987).

5A3. Rat immunocytoma (RIC).

The rat immunocytoma (RIC) is a plasma cell tumor which develops spontaneously in approximately 30% of the rats of the LOU/Ws1 strain (for review see Bazin *et al.*, 1988; Sümegi *et al.*, 1987). The tumors originate in the ileocecal lymph nodes, and approximately 60% of the tumors are Ig secreting. Significantly, unlike the mouse plasmcytoma, it is very rare to see IgA producing tumors but approximately 50% of the tumors secrete IgE, and various γ isotypes are also secreted. The reciprocal 6:7 translocation has been identified in all sixteen tumors examinated (Pear *et al.*, 1986a; Pear *et al.*, 1988a; Wiener *et al.*, 1982). In 12 out of 14 tumors c-*myc* rearrangement was seen in a 40 kb region surrounding the gene. Analysis of the sequence juxtaposed to the c-*myc* show that IgH switch regions are the targets in at least five tumors and that there is strong correlation between the secreeted Ig and the c-*myc* target (Pear

et al., 1988a). The breakpoints are clustered in a 1.5 kb region upstream of the proximal promoter. This is unlike the situation in MPC and sporadic BL where most of the breakpoints are seen in intron 1.

In the IR75 we have found complicated translocation, where the c-*myc* is juxtaposed head-to-head to the switch μ region, and then behind it is an inversion of regions derived from upstream of $\gamma 1$ (paper IV). A similar molecular geometry was found in an exceptional MPC (Fahrlander *et al.*, 1985). Basically, this tumor showed us that similar to the mouse plasmacytoma and BL, the RIC translocation juxtaposes the c-*myc* with IgH sequences.

Cloning of the rearranged c-*myc* genes from four tumors, IR50, IR75, IR162, and IR223, has shown that the c-*myc* and IgH sequences are rearranged head to head (Pear *et al.*, 1988b; Tian and Faust 1987; paper IV). These results are similar to the findings with BL and MPC (Cory, 1986).

5A4. The *PVT* locus.

The *pvt* -1 locus in mouse, *PVT* in human, and *Mis* -1/*Mlvi* -1 in rat are homologous, map to the same chromosome as the c-*myc* gene (paper V; Graham and Adams, 1986; Koehne *et al.*, 1989) and are located 3´ of it (Banerjee *et al.*, 1985; Wirschubsky *et al.*, 1985). The *PVT* in human has been shown to be transcriptionally active and is located from 57 kb to at least 253 kb downstream of c-*myc* (Henglein *et al.*, 1989; Shtivelman *et al.*, 1989; Shtivelman and Bishop, 1989). The *PVT* is dispersed and the first intron spans over 50 kb. In the variant translocation of BL (t(2;8) and t(8;22)) and variant translocation of MPC (t(6;15)) involving one of the immunoglobulin light chains the molecular breakpoint is located within the *pvt* -1 region (Banerjee *et al.*, 1985; Shtivelman *et al.*, 1989; Webb *et al.*, 1984). Furthermore, the *pvt* -1/*Mis* -1 is the proviral insertion site of virally-induced mouse and rat lymphomas (Graham *et al.*, 1985; Jolicoeur *et al.*, 1985; Lemay and Jolicoeur, 1984; Villeneuve *et al.*, 1986). It has been claimed that the proviral integration loci *Mlvi* -1 and *pvt* -1/*Mis* -1 represent two different loci but recent evidence shows that they are identical (Koehne *et al.*, 1989).

C-*myc* expression is elevated in variant MPCs and in T-cell lymphomas with retrovirus insertion in the vicinity of *pvt* -1. Therefore, it has been speculated that the *pvt* -1 is involved in the control of c-*myc* expression by influencing superchromatin structure. Still, the detection of a transcriptionally active gene at the *PVT* locus suggests that the transcriptional control of the two genes, c-

myc and *PVT*, is propably more complicated than that, especially in the context of a possible cross-regulation between them (Shtivelman *et al.*, 1989).

5B. Gene amplification.

Somatic amplification of specific genes have been implicated in a variety of adaptive responses of cells to environmental conditions since the discovery in drug-resistant eukaryotic cells (Schimke, 1982). In a similar way, the amplification of *myc* genes in many different tumor cells is thought to give some growth advantage to the cell, in view of the documented connection between the *myc* gene and cellular growth control (see chapter 9).

The first report of a somatic amplification of the c-*myc* was in a promyelocytic leukemia cell line HL-60 (Collins and Groudine, 1982; Dalla-Favera *et al.*, 1982b). Of interest is that this cell line can be differentiated to different cell types by either DMSO or TPA/RA/VitD, resulting in downregulation of the c-*myc* gene (Reitsma *et al.*, 1983; Westin *et al.*, 1982a). The c-*myc* is also amplified in some plasma cell leukemias (Sümegi *et al.*, 1985) and ANLL (Alitalo *et al.*, 1985; Asker *et al.*, 1988). Since these are highly malignant form of human leukemias, and in the view of *myc* amplifications in more malignant forms of SCLC and neuroblastoma (see later), the c-*myc* amplification in leukemias may promote the clonal evolution of a more highly malignant cell variant.

In SCLC the c-*myc*, N*myc*, or L*myc* are amplified and/or expressed in 60% of cell lines tested (Brooks *et al.*, 1986; Little *et al.*, 1983), suggesting certain equivalence of the *myc* family members. The c-*myc* amplification correlates with the more malignant behavior of the variant cell histology of SCLC (Brooks, *et al.*, 1986). Thus, c-*myc* amplification appears to be related to the tumor progression rather that initiation. The B*myc* has not been found amplified in 40 different SCLC (unpublished observation).

In a similar way the N*myc* is found amplified in neuroblastoma and is strongly correlated with advanced stages (III-IV); about 50% of these show evidence of amplified N*myc*, while no amplifications are found in stage I and II tumors (Brodeur *et al.*, 1984). The prognosis for patients at stage III and IV is usually very poor; 2 year survival is 10-30%, compared with 75-90% for stages I and II (Pochedly, 1982). Seeger *et al.*, (1985) reached a similar conclusion, namely that amplification of N*myc* was associated with the worst prognosis. The estimated progression free survival at 8 months was 70%, 30%, and 5% for

patients whose neuroblastomas had 1, 3-10, or more than 10 N*myc* copies, respectively. In addition to amplification of *myc* family genes an interesting analogy of the HL60 and neuroblastoma cells is that both harbor point mutations within the N*ras* locus, accounting for their ability to transform NIH3T3 cells (Bos *et al.*, 1984; Murray *et al.*, 1983; Taparowsky *et al.*, 1983).

Several reports have demonstrated c-*myc* amplification in 6-30% of cases of breast cancer (Escot *et al.*, 1986; Guérin *et al.*, 1988; Kozbor and Croce, 1984). Some of these reports claim that overexpression of the c-*myc* gene correlates with poor prognosis (Guérin *et al.*, 1988) and tumorigenicity (Lavialle *et al.*, 1988).

Cells carrying amplified DNA either as a homogeneously staining region (HSR) or as double minute (DM) often grow more slowly unless the dosage of the amplified genes provides a growth advantage, and in the absence of such a selective pressure DM are lost (Schimke *et al.*, 1980). The polyomavirus induced mouse tumor cells, SEWA, contains DM which increase in number when injected into the mouse ascites and are reduced when the tumor cells are explanted back to tissue culture (Levan *et al.*, 1981). It has been shown that the DM in SEWA are the sites of amplified c-*myc* copies and that the tumors contain enhanced levels of c-*myc* mRNA and protein (Schwab *et al.*, 1985b). The c-*myc* amplification is closely associated with ascites growth of the SEWA, since the amplification is lost when the tumor was grown and serially transplanted as solid tumor (Minarovits *et al.*). The degree of c-*myc* amplification in SEWA correlates with the tumorigenicity, detected by subcutaneous inoculation in syngeneic mice (Martinsson *et al.*, 1988).

Still another tumor with c-*myc* amplification is COLO320, a colon carcinoma. This amplification is interesting in several aspects. The c-*myc* is amplified either in DM chromosomes or as a HSR, in a truncated form, lacking the first exon, or as an intact gene, respectively (Alitalo *et al.*, 1983). This tumor was the first one to be used to show that truncated forms of c-*myc* mRNA have shorter half life than the normal size counterpart (Rabbits *et al.*, 1985). Furthermore, a correlation between the *PVT* locus and c-*myc* amplification has been suggested since the first exon of *PVT* has been found transposed to the first intron of the c-*myc* in the COLO320 (Shtivelman and Bishop, 1989).

5C. Viral transduction and viral insertions

RNA tumor viruses can be either acute or slow transforming (or chronically acting). The former category of viruses has transduced a mutated cellular transforming sequences and induces tumors after short latency periods by virtue of a viral oncogene (v-*onc*). These viruses are replication defective and need a competent helper virus of the slow transforming type for the replication. The avian leukemia viruses carrying the v-*myc*, commonly called the myelocytomatosis viruses, have broad pathogenic spectrum and induce carcinomas, endotheliomas, and sarcomas in addition to the characteristic leukemic disorder called myelocytomatosis (Beard, 1980). The myeolocytomatosis viruses are able to transform fibroblasts and macrophages into a malignant phenotype (Bishop, 1983).

The chronically acting viruses are replication-competent and do not carry an oncogene in their genome, but rather cause tumors after a long latency period through the activation of cellular oncogenes by integration in their vicinity (for review, see Nusse 1986). Provirus insertion in the neighborhood of c-*myc* has been implicated as important in the development of avian leukosis virus (ALV) and murine leukemia virus (MuLV)-induced tumors (for review see Clurman and Hayward, 1988; Nusse and Berns, 1988). The bursa of Fabricius is the target site for tumors induced in chickens susceptible to ALV-induced B-cell lymphomas, with the provirus integration adjacent to the c-*myc* (Hayward *et al.*, 1981, Payne *et al.*, 1982), thought to be a causative factor in the initiation of oncogenesis. The provirus integrates mostly in the first intron of c-*myc*, and the gene is transcriptionally activated. The c-*myc* gene can be activated by transcription from the viral promoter, in the case where the provirus is mutated (Cullen *et al.*, 1984) or by enhancer function (Linal and Groudine, 1985).

A similar mechanism of activation has been described in the case of virally induced murine T-cell lymphomas, although the promoter insertion is less frequent (Corcoran *et al.*, 1984; Li, *et al.*, 1984; O´Donnel *et al.*, 1985; Selten *et al.*, 1984). It is of interest that the breakpoints correlate with the integration points in tumors with activated c-*myc* by chromosomal translocation; 5´ of the coding region or 3´ in the *pvt* -1/*Mis* -1/*Mlvi* -1 locus.

It has been shown that 35% of primary MuLV-induced murine T cell lymphomas carry proviral insertion in the 3´ noncoding part of N*myc*, whereas proviral insertion near c-*myc* was found in 40% of the tumors (VanLohuizen *et al.*, 1989a, b), supporting the widely accepted importance of *myc* activation in lymphoid neoplasias.

6. TRANSFORMATION BY *MYC*.

There is a large body of evidence suggesting that progression to malignancy is a multistep process (Foulds, 1958; for review, see Weinberg, 1985). The *myc* (s) can complement activated *ras* genes in inducing the neoplasmic transformation of early-passage cells or primary cell types, while either oncogene alone is essentially nontransforming. *Myc* and *ras* are considered to be representative of two classes of oncogenes that encode nuclear or cytoplasmic proteins, respectively, and cooperate in the transformation of primary cells. Thus, the c-*myc* gene has been linked to immortalization activity (Mougneau *et al*., 1984), while the action of Ha-*ras* has been correlated with transformation activity (Land *et al*., 1983). Additional studies, however, indicate that c-*myc* expression constructs can induce transformation in immortalized cell lines (Keath *et al*., 1984a; Kelekar and Cole, 1986) and that Ha-*ras* can immortalize primary cells (Spandidos and Wilkie, 1984). Currently, it is not clear how the activities of these genes result in the observed spectrum of deregulated growth, but tumorigenic conversion of REFs by single transfected *myc* or *ras* gene appears to require special culture conditions and high levels of gene expression (Land *et al*., 1986). Complementation between *myc* and *ras* holds for an increasing number of cell types (Alexander *et al*., 1989; Ridley *et al*., 1988; Schwartz *et al*., 1986; Sinn *et al*., 1987). Similarly, it has been shown in a reconstituted mouse prostata gland, that v-*ras* and v-*myc* can induce separately specific types of premalignant lesions and that the step-like progression of tumors from benign to increasingly aggressive stages could be caused by activation of both genes (Thompson *et al*., 1989).

Like the c-*myc* .gene, the N*myc* and L*myc* genes can cooperate with mutant Ha-*ras* gene to cause malignant transformation of REFs, as determined by morphology, soft agar cloning, and tumorigenicity (Birrer *et al*., 1988; DePinho *et al*., 1987b; Schwab *et al*., 1985a; Small *et al*., 1987; Yancopoulos *et al*., 1985), while the B*myc* role remains undetermined. A bacterial N*myc* fusion protein has been shown to antagonize the N*myc* acitvity by reducing the foci formation of REF cells, cotransfected with activated Ha-*ras* gene (Ueno *et al*., 1988). Furthermore, it has been demonstrated that rat embryo cells transfected with N*myc* increases the generation number showing that enhanced expression of N*myc* can rescue normal cells from senescence (Schwab and Bishop, 1988).

Transgenic mouse models have shown directly that constitutive c-*myc* or N*myc* expression promotes lymphoid neoplasia *in vivo* (Adams *et al*., 1985; Alexander *et al*., 1989; Dildrop *et al*., 1989; Rosenbaum *et al*., 1989), as well as

c-*myc* in some other tumor growth (Adams *et al.*, 1985; Leder *et al.*, 1986; Sinn *et al.*, 1987). Similar cooperativity between the *myc* and *ras* oncogene has been found in transgenic mice, as well as cooperativity of *myc* and v-*raf* (Alexander *et al.*, 1989; Sinn *et al.*, 1987). Still other oncogenes that can cooperate with *myc* in the transforming activity are the v-*src* (MacAuley and Pawson, 1988) and v-*abl* (Ohno *et al.*, 1984).

7. TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION OF *MYC* EXPRESSION.

The c-*myc* oncogene is normally subject to complex regulation at the transcriptional and post-transcriptional levels in proliferating and differentiating cells. It is activated in responce to growth stimuli and generally, though not always, repressed in response to differentiation signals. The c-*myc* mRNA levels are rapidly increased or decreased by either or both of two rapid mechanisms; regulation of mRNA elongation and of mRNA stability.

Eick and Bornkamm (1986) proposed that two conformations may be formed in the 5´ part of the first exon; a readthrough conformation with one stem and loop, and an alternative conformation with two stems and loops followed by a polyU stretch, which might serve as a transcription termination signal. Regulation by attenuation, in *E.coli* as well as in SV40, involves a small protein encoded by the RNA leader that stabilizes one of both mRNA conformations (Hay and Aloni, 1985; Yanofsky, 1981). The analogy between the different systems has reemphasized the search for proteins which may eventually be encoded by the reading frame in the first exon (Gazin *et al.*, 1984; Gazin *et al.*, 1986), and by the upstream reading frame present in c-*myc* RNA initiated at P_0 (Bentley and Groudine, 1986b). Similar transcription block has been described in the L*myc*, but it is not detected in the N*myc* gene (Krystal *et al.*, 1988).

As discussed earlier a "dehancer activity" is found 5´ of the c-*myc* gene (section 5A1) and post-transcriptional regulation at the level of mRNA stability is thought to be localized in the noncoding first exon and the noncoding part of the third exon (section 4B1). The problem with *myc* half-life is to find a "feature" of the molecule that makes *myc* mRNA have a short half-life. Such a feature must somehow be encoded in the sequence, but it is not necessarily a single motif. In fact, for *myc* it is clear that several sites have a combined effect to maintain the normal half-life. The 5' exon is a necessary but not sufficient stability determinat (if removed the half-life goes up, if put on another gene, nothing happens) (Pei and Calame, 1988), while the 3' noncoding region is

necessary for normal *myc* half-life and sufficient to change the half-life of another mRNA if added to it (Jones and Cole, 1987). Both are stability determinants, but of different quality. How they interact is still not clear. There are several theoretical possibilities; a direct secondary structure interaction forming an attack site for a specific RNAse, and indirect effect through altered translational efficiency, etc. The 3' noncoding region thus has a "destabilizer", but that is not the whole explanation of the normal short c-*myc* half-life.

The transcriptional situtation in the c-*myc* locus is further complicated by the finding of antisense transcription in the region upstream of P2, a phenomenon the biological significance of which is unclear (Bentley and Groudine, 1986b; Nepveu and Marcu, 1986).

Several transactivators have been suggested to influence c-*myc* transcription. The *jun/fos* protein complexes seem to regulate gene expression and one of the target candidates for such regulation is the c-*myc* oncogene (Hay *et al.*, 1989). The human c-*myc* promoter is the first cellular target described for transactivation by E1a, mediated by E2F nuclear factor (Sassone-Corsi and Borelli, 1987; Thalmeier *et al.*, 1989). Furthermore, a tissue specific regulation of c-*myc* expression by steroid hormones has been reported (Fink *et al.*, 1988).

8. CHARACTERIZATION OF *MYC* PROTEINS.

The sequences in the coding regions of the *myc* genes have significant similarities, suggesting that they may serve similar but distinct physiological functions. The c-*myc*, N*myc*, and L*myc* encode nuclear phosphoproteins (Alitalo *et al.*, 1983; DeGreve *et al.*, 1988), which bind DNA *in vitro* (Donner *et al.*, 1982; Evan *et al.*, 1988; Evan *et al.*, 1989; Persson *et al.*, 1984a; Ramsay *et al.*, 1986; Slamon *et al.*, 1986). The c-*myc* has been shown to be phosporylated by casein kinase II, in regions of functional importance (Lüscher *et al.*, 1989). The c-*myc* and N*myc* proteins bind to different structures within the nucleus (Evan *et al.*, 1986; paper VI). The c-*myc* protein can be extracted from the nucleus with high salt concentration, while 30% of the N*myc* protein remains in the nucleus (Evan *et al.*, 1986). Transfection of CV-1 cells by SV40 based vectors with high levels of c-*myc* or N*myc* protein expression, suggests that the two proteins bind to different structures within the nucleus (paper VI). In c-*myc* transfected cells the overproduced protein product accumulates in large amorphous globules that displace the normal chromatin and do not stain for DNA. In N*myc* transfected cells condensed chromatin loops are formed.

The c-*myc*, N*myc*, and L*myc* genes produce several proteins of different sizes. The c-*myc* produces two major proteins of the sizes 64 kd and 67 kd (p64 and p67) and a minor protein of the size 46 kd (Lüscher and Eisenman, 1988). The initiation of the p64 is in the second exon from a typical AUG initiation of translation, while the p67 starts in the first exon from a CUG codon (Hann *et al.*, 1988). The size difference of the two proteins is additional 14 amino acids in p67, at the N-termini. The p67 is lost in many Burkitt lymphomas. Still, the extra 14 amino acids at the amino terminal are not highly conserved between species (Bernard *et al.*, 1983; Hayashi *et al.*, 1987), and are not found in the putative B*myc* peptide (paper III).

The N*myc* gene shows 4 different sized proteins in SDS-PAGE electrophoresis, of sizes p67/p65 and p62/p64 (Mäkelä *et al.*, 1989). The p67/p65 disappears by phosphatase treatment, suggesting that they are phosphorylated forms of the p62/p64. The p62 and p64 differ in the N-termini; the larger form has additional 8 amino acids, and both proteins initiate from AUG codon in the 5′ part of the second exon.

In similar experiments the L*myc* has three bands sized 60-66 kd that convert to one band at 60 kd after phosphatase treatment (Saksela *et al.*, 1989). Based on *in vitro* translation DeGreve *et al.*, (1988) reached a different conclusion; that the different forms of L*myc* protein arise from differential RNA splicing coupled to differential initiation of translation. The fully processed 3.6 kb mRNA synthesizes only the p60 form, while the 3.9 kb mRNA (containing intron 1) could translate both the p60 and p66 forms (DeGreve *et al.*, 1988). Therefore, alternative processing of intron 1 provides a mechanism for determining the level of template available to translate the p66 from *in vivo*. This possibility is particularly interesting if the two L*myc* protein forms have different functional properties in growing cells, as has been suggested for the two c-*myc* proteins (Hann *et al.*, 1988). In addition to this there are three L*myc* bands at 32-37 kd, which also turn into one band as a result of dephosphorylation (Saksela, *et al.*, 1989), and are suggested to be encoded from alternatively processed mRNA, and lack the 3rd exon sequences (Kaye *et al.*, 1988; DeGreve *et al.*, 1988). This shorter form of L*myc* protein is localized in the cytoplasm (Ikegaki *et al.*, 1989), suggesting a different role compared to the full size nuclear protein. No protein has yet been detected from the B*myc* gene *in vivo*, but sequence homology to c-*myc* coding region seems to be limited to the second exon, while third exon sequences are lacking in the B*myc* (paper III).

Like the mRNA species of the *myc* family members (Dani *et al.*, 1984), their protein products are unstable. The c-*myc* and N*myc* proteins have a very short half life, 30-40 minutes, while the L*myc* protein is a little more stable, with a half life of 90 minutes (Evan *et al.*, 1988; Evan *et al.*, 1989). A protein sequence rich in prolin, glutamate, serin and threonine (the so-called "PEST" sequence) is thought to be involved in the destabilization of the c-*myc* protein (Rechsteiner *et al.*, 1987).

Comparison of the coding DNA sequences of the different *myc* genes, show that several regions have high homology, the so-called "*myc* -boxes". Nine such regions have been described. C-*myc* and N*myc* have seven such boxes in common (Kohl *et al.*, 1986), c-*myc* and L*myc* five boxes, N*myc* and L*myc* seven boxes (Legouy *et al.*, 1987) and B*myc* two boxes found in all the other *myc* family members and an additional box found in c-*myc* and N*myc* (paper II; paper III). In addition to these boxes the B*myc* has high homology to c-*myc* in other regions.

A functional association has been suggested between the c-*myc* protein and DNA replication (Classon *et al.*, 1987; Iguchi-Ariga *et al.*, 1987). Other data suggest a role for the c-*myc* protein in regulation of gene expression at the transcriptional level (Kaddurah-Daouk *et al.*,1987; Kingston *et al.*, 1984; Onclercq *et al.*, 1988), and possibly in RNA processing (Prendergast and Cole, 1989; Sullivan *et al.*, 1986). The effects of the c-*myc* protein on transcription has been shown to be both negative and positive (Kaddurah-Daouk *et al.*, 1987) and the larger protein (p67) that initiates in the first exon seems to be important in this aspect (Onclercq *et al.*, 1988). The c-*myc* protein has been found associated with small nuclear ribonucleoproteins (RNPs), suggesting a possible role in RNA processing or transport (Spector *et al.*, 1987). It has also been suggested that the c-*myc* protein is regulating gene expression at a post-transcriptional level, by other mechanisms than stability of cytoplasmic mRNA (Prendergast and Cole, 1989). These findings are not necessarily contradicting, one study suggests that the *myc* protein has a double function, as a transcriptional and DNA replication factor (Iguchi-Ariga *et al.*, 1988) or alternatively the effects of the c-*myc* protein to enhance DNA replication are not necessarily direct, but could act via secondary gene expression (Classon *et al.*, 1987). In fibroblasts with high levels of N*myc* protein, a higher rate of DNA synthesis has been reported (Cavalieri and Goldfarb, 1988). The cellular genes that c-*myc* possibly alters the expression of are still unknown. Two dimensional electrophoresis showed that c-*myc* activated expression of eight cellular proteins while five are downregulated, and a Go/G_1 specific cDNA is

also affected by c-*myc* (Scweinfest et al., 1988). An other candidate is the mr1 gene (Prendergast and Cole, 1989), that has 85% homology to human plasminogen activator inhibitor-1, a regulator of extracellular protease activity, which is implicated in tissue remodeling, tumor invasion, and metastasis (Prendergast, personal communication). Two cellular specific genes, H1 histones, are shown to be negatively regulated by c-*myc* and the timing of their induction suggests that they may play an important role in achieving commitment to terminal differentiation (Cheng and Skoultchi, 1989). Another putative target of c-*myc* is expression of ornithine decarboxylase (Dean *et al.*, 1987), a highly regulated enzyme of the biosynthetic pathway of polyamines in mammalian cells (Pegg and McCann 1982; Tabor and Tabor, 1982). Interestingly, it has been shown that the functional ornithine decarboxylase gene has been rearranged with the immunogloulin heavy chain locus in mouse myeloma cells, in a c-*myc* like manner (Katz and Kahana, 1989).

9. *MYC* EXPRESSION IN RELATION TO CELL PROLIFERATION AND DIFFERENTIATION.

The c-*myc* expression is closely associated with the proliferative state of many mesenchymal cells. Most of our knowledge about the role of *myc* genes in growth control comes from systems in which the transition from quiescence to proliferation and eventually back to growth arrest can easily be manipulated.

It is well documented that c-*myc* expression is induced by mitogenic stimuli (mitogens and growth factors) in a variety of cell types and that cells expressing high levels of exogenous c-*myc* escape from growth factor requirement. Mitogenic stimuli, such as lectins, peptide growth factors, phorbol esters, calcium ionophores, antireceptor antibodies, lymphokines, and partial hepatectomy, commonly cause marked, transient elevations in c-*myc* mRNA and protein levels in normal cells by a variable combination of increased transcription and decreased breakdown (Armelin *et al.*, 1984; Blanchard *et al.*, 1985; Campisi *et al.*, 1984; Conscience *et al.*, 1986; Coughlin *et al.*, 1985; Dean *et al.*, 1986a; Goyette *et al.*, 1984; Greenberg and Ziff, 1984; Kelly et al., 1983; Lacy *et al.*, 1986; Makino *et al.*, 1984; Mehmet *et al.*, 1988; Persson *et al.*, 1984b; Persson *et al.*, 1985; Ran *et al.*, 1986; Reed *et al.*, 1985a, b; Smeland *et al.*, 1985). The c-*myc* expression is induced by serum, platelet-derived growth factor, fibroblast growth factor and the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate in BALB/c3T3 fibroblasts and by Concanavalin A and

lipopolysaccharide in T and B lymphoid cells, respectively (Kelly *et al.*, 1983). Resting human peripheral blood mononuclear cells exhibit a biphasic *myc* activation pattern in response to the lectin mitogen phytohemaglutinin with peaks at 1-2 h and 24 h (Reed *et al.*, 1986).

Fibroblastic cells responsive to epidermal growth factor and the combined action of other purified growth factors such as thrombin and insulin also activate c-*myc* expression as an early growth response (Blanchard *et al.*, 1985; Liboi *et al.*, 1986). Addition of serum to serum-deprived fibroblasts, antigenic or mitogenic stimulation of lymphocytes (Kelly *et al.*, 1983), and partial hepatectomy (Goyette *et al.*, 1984; Makino *et al.*, 1984) lead to an up to hundredfold increase in c-*myc* mRNA with a maximun after 1-2 h, well before the cells enter S phase. The levels of c-*myc* are controlled by the action of growth factors, and constitutive expression of the gene in some fibroblast systems partially relieves dependence on these factors for entrance into the cell cycle (Armelin *et al.*, 1984; Cavalieri *et al.*, 1987; Sorrentino *et al.*, 1986; Stern *et al.*, 1986). In line with a biologically significant function of the increased c-*myc* expression seen when quiescent cells reenter the cell cycle is that injection of purified c-*myc* protein into cell nuclei leads to the onset of DNA synthesis when cells are subsequently exposed to plasma (Kaczmarek *et al.*, 1985). Although c-*myc* mRNA levels change dramatically when quiescent cells reenter the cell cycle, c-*myc* expression is invariant throughout the cell cycle (Hann *et al.*, 1985; Thompson *et al.*, 1985). It has been shown that enforced expression of c-*myc* precludes entry into a distinct predifferentiation state in G_0/G_1 that is a prerequisite for terminal differentiation, and therefore, it was proposed that c-*myc* is a molecular switch directing cells either for a pathway that can lead to continued proliferation or to terminal differentiation (Freytag, 1988). *Myc* expression is also induced as an early response to EGF or nerve growth factor in the rat pheochromocytoma cell line PC12 (Greenberg *et al.*, 1986).

C-*myc* expression can be downregulated by interferon (INF). Addition of IFN-β or α to Daudi BL cells causes them to arrest at the G_0/G_1 phase of the cell cycle and to down-regulate c-*myc* expression at the transcriptional or post-transcriptional level (Dani *et al.*, 1985; Einat *et al.*, 1985). A β-related IFN is produced by U937 human histiocytic lymphoma cells induced to differentiate by phorbol myristate acetate (PMA) and also in M1 murine myeloid cells treated with natural differentiation iducers derived from mouse lung-conditioned media (Resnitzky *et al.*, 1986)). This IFN-β appears to function as an autocrine growth inhibitor resulting in G_0/G_1 arrest as well as c-*myc* suppression (Resnitzky *et al.*, 1986). Antibodies against type I IFN that were added along with

differentiation inducers prevented most of the loss in cell growth rate and also resulted in a smaller reduction in *myc* RNA levels (Resnitzky *et al.*, 1986).

Deregulated c-*myc* expression can block the differentiation of several cell types. In a number of cell systems, cycling of the cells and induction of differentiation appear to be mutually exclusive. In the best studied systems of *in vitro* differentiation, mouse erythroleukemia cells (MEL), and the human monocytic leukemia cell line HL60, c-*myc* mRNA decreases rapidly after addition of inducers of differentiation (Reitsma *et al.*, 1983; Westin *et al.*, 1982). At least in MEL cells, the c-*myc* decrease is biphasic with an intermittent increase after about 18 h (Lachman and Skoultchi, 1984). High constitutive expression of c-*myc* blocks the differentiation of MEL cells into cells that resemble mature erythrocytes (Coppola and Cole, 1986; Dmitrovsky *et al.*, 1986; Prochownik and Kuwowska, 1986).

As a general conclusion it can be said that proliferating cells of all kinds express one of the *myc* -family members as long as they grow, but tend to down-regulate it when cells have to leave the cycling compartment. Terminal differentiation can be prevented by introducing constitutively expresed *myc* -genes and , contrariwise, *myc* -antisense can induce differentiation (Prochownik *et al.*,1988; Wickström *et al.*, 1988). These observations suggest that c-*myc* plays a central role in the regulatory networks that control cellular proliferation and differentiation. The absence of *myc* transcription in resting cells, negative correlation with cell differentiation, and presence in all proliferating cells is in line with the conclusion that the pathological activation of the *myc* by chromosomal translocation or viral insertion prevents the cell from leaving the cycling compartment when it is programmed to do so.

In the case of retinoic acid (RA) induced differentiation of human neuroblastoma (NB) cells, a decresed level of N*myc* expression is detected within 6 h of treatment and precedes both cell-cycle changes and morphological differentiation (Amatruda *et al.*, 1985; Hammerling *et al.*, 1987; Horii *et al.*, 1989; Thiele *et al.*, 1985). Furthermore, it has been shown that constitutive expression of N*myc* blocks RA induced differentiation but not growth arrest of human NB cell lines (Thiele, personal communication).

The expression of L*myc* in correlation to cell differentiation is poorly studied, mainly since no good inducible cell system is available. It has been shown that a transfected L*myc* gene can substitute for c-*myc* in blocking MEL differentiation (Birrer *et al.*, 1989). We have shown that L*myc* is downregulated during RA induced differentiation of embryonal carcinoma (EC) cells (paper VIII).

Table 3. mRNA expression of *myc* family members in F9 embryonic carcinoma cells (paper VII; paper VIII).

F9-mRNA levels	c-myc	Nmyc	Lmyc	Bmyc
Retinoic acid	↓	↓	↓	1x
Serum starv.	1/10x	1/10x	1/10x	1x
Ins.+Transf.	5x	1x	1x	1x
Cycloheximide	2x	1x	1.7x	1.3x
ActD ($t_{1/2}$)	40′	120′	30′	340′

We have studied the expression pattern of *myc* family genes in F9 and PCC7 EC cells (Table 3; paper VII; paper VIII). The F9 and PCC7 were induced to differentiate to visceral endoderm and nerve like tissue, respectively. By "run on" analysis on PCC7 cells we have shown that c-*myc* is repressed at the transcriptional level, but these cells express N*myc*, which is down-regulated during differentiation, by a post-transcriptional mechanism.

The F9 cells express all four *myc* family members at the mRNA level (Table 3). The c-*myc*, N*myc*, and L*myc* genes are down-regulated during RA induced differentiation to visceral endoderm, while B*myc* is expressed constitutionally at low level (paper VII; paper VIII). C-*myc* and N*myc* are also down-regulated during formation of parietal endoderm from F9 cells (Dean *et al.*, 1986b; Dony *et al.*, 1985; Griep and DeLuca, 1986; Jakobovits *et al.*, 1985). As a further confirmation of the c-*myc* role in differentiating F9 cells it has been shown by using antisense *myc*, that down-regulation of c-*myc* is sufficient and necessary for F9 cell differentiation (Griep and Westphal, 1988). Overexpression of exogenous c-*myc* gene is thought to interfere with early events in F9 cell differentiation (Onclercq *et al.*, 1989)

Similarly, the c-*myc*, N*myc*, and L*myc* genes are down-regulated during serum starvation while B*myc* is unaffected. These findings are in line with constitutive expression of B*myc* in devloping rat tissues in contrast to the more limited expression of other *myc* family members (paper II; paper VIII). c-*myc*

is the only *myc* family member that can be induced in F9 cells by insulin/transferrin, suggesting stronger correlation to the mitogenic stage of the cells than the other *myc* genes. L*myc* is poorly analysed in this aspect, but it has been suggested that alteration of protein phosphorylation is followed by TPA or serum activation of small cell lung carcinoma cell line (Saksela *et al*., 1989).

The c-*myc*, L*myc*, and B*myc* genes are upregulated in F9 cells when protein synthesis is blocked by cycloheximide, suggesting that the expression of these genes are under the control of labile proteins. A cytoplasmic destabilizer, possibly a labile ribonucleoprotein, has been shown to regulate c-*myc* mRNA stability *in vitro* (Brewer and Ross, 1989). The half life of the mRNA in F9 cells is short for c-*myc* and L*myc*, 40 and 30 min., respectively. The N*myc* mRNA is more stable, with a half life of 2H, and even longer for B*myc*, 6H.

10. CONCLUSIONS

The nuclear products of the c-*myc* and N*myc* genes bind to different subnuclear structures.

Similar to MPC and BL, the RIC translocation juxtaposes the c-*myc* with IgH sequences. Although the exact mechanism of c-*myc* activation may vary between different tumors, its occurrence appears to be essential in the tumorigenic pathway of RIC, MPC, and BL. As the c-*myc* and *pvt* -1 are synthenic in human, mouse, and rat, a large chromosomal region around c-*myc* is conserved in mammals during evolution.

The *myc* family members are dispersed over the rat and mouse genome, as already shown for humans, and localize on different chromosomes.

B*myc* has high homology to the 5´ part of c-*myc* but no homology to the coding part of the third exon. Cloning of cDNAs with different 3´ noncoding exons suggest alternative mRNA processing of the B*myc* gene.

B*myc* is a constitutively expressed gene, expressed in many tissues, both at fetal and adult stages. C-*myc*, N*myc*, L*myc*, and B*myc* expression is differentially regulated in developing tissues and in embryonic carcinoma cells in response to serum deprivation, growth factors, and/or induction of visceral endoderm differentiation.

11. ACKNOWLEDGEMENTS

This work has been done at the Department of Tumor Biology, Karolinska Institutet, Stockholm, Sweden. It has been a priviledge to work here and I would like to thank everybody that has contributed in some way.

George Klein for the opportunity to work at the Tumor Biology, his guidance, interest, support, and fruitful ideas.

Janos Sümegi who introduced me to various technics in molecular biology and for the continuous support, criticism and advice.

My collaborators on the *myc* family genes; Marie Classon, Marie Henriksson, Pei Jin, Warren Pear, Thomas Sejersen, Gabor Szabo, Johan Thyberg, and Zvi Wirschubsky.

My "B*myc* " collaborators; Kenth Andersson, Charlotte Asker, Håkan Axelson, and Michael Steinitz,

Other members of the Molecular Biology group: Peter Berglund, Ferenc Boldog, Javier Castresana, Rikard Erlandsson, Zoltan Marcsek, Chinmay Panda, Alan Pitt, Lazlo Szekely, Ingrid Törnberg, Eva Uzvölgyi, Tao Wen, and Klas Wiman.

Ruth Klöcker and others who have laboured with the breeding, feeding and everyday care of the animals participating in this study.

The secretaries; Elsbeth Bengtsson, Kerstin Blomqvist, Marie Bohm, Gudrun Eriksson, Marie-Louise Eschricht, Marta Nilsson, Ann Schmalholz, Ann-Sofie Hollstein Yamamoto, as well as Zofia Christiernin, Alex Feldötö and Janos Kovacs for all their help.

Other students, researchers, and technicians; Javier Avila, Maria Teresa Bejarano, Tina Dalianis, Barbro Ehlin-Henriksson, Ingemar Ernberg, Kerstin Falk, Mona Hedenskog, Eva Klein, Karin Kvarnung, Klas Kärre, Nongnit Levin, Hans Gustaf Ljunggren, Maria Grazia Masucci, Janos Minarovits, Mai Paulsson, Torbjörn Ramqvist, Håkan Randahl, Anders Rosén, Agneta Sandlund, Santiago Silva, Pankaj Trivedi, Anders Wennborg, Francis Wiener, Gösta Winberg, and Lars Ährlund-Richter.

My collegues and co-authors who provided fruitful scientific collaboration; Hervé Bazin, Uta Francke, Päivi Koskinen, Maximilian Münke, Göran Levan, Magnus Rahm, David Steffen, Sanjoy Sundaresan, and Josiane Szpirer.

Valgardur Egilsson for introducing me to science.

Agnetha Viklund, Anton Helgi Jónsson, Árni Thór Sigurdsson, Björg Pétursdóttir, Björn Thorsteinsson, Fridgeir Baldursson, Karólína Hlíddal, Lárus Valur Lárusson, Magnús Einarsson, Magnús Ingimundarson, Margrét Sveinsdóttir, Rikardo Olivos, Rossana Mancini, Steinunn Helgadóttir, and Thórdur Einarsson, for friendship and pleasant company during my years in Stockholm. Special thanks to Sigurbjörg Thorsteinsdóttir for all her help during the early days at Karolinska.

Stefan Magnusson for taking care of my children while I was in the lab.

My mother and sister for care.

My wife, Tóta, and my children, Lárus, Tinna, and Valur, for love, inspiration, patience, and for giving me the joy of life.

The studies in this thesis were supported by; Public Health Service grant number 3R01CA140554 awarded by the National Cancer Institute, Department of Health and Human

Services; the Swedish Cancer Society; the Swedish Medical Research Council; Hedlunds Stiftelse; the funds of Karolinska Institutet; the Concern Foundation, Los Angeles; Nordiska Forskarstipendier; The Swedish Institute; and Svenska Sällskapet för Medicinsk Forskning.

12. REFERENCES

1. Adams, J.M., Gerondakis, S., Webb, E., Corcoran, L.M., Cory, S. 1983. Cellular *myc* oncogene is altered by chromosome translocation to an immunoglobulin locus in murine plasmacytomas and is rearranged similarly in human Burkitt's lymphomas. *Proc. Natl. Acad. Sci. USA* **80,** 1982-1986.
2. Adams, J.M., Harris, A.W., Pinkert, C.A., Corcoran, L.M., Alexander, W.S., Cory, S., Palmiter, R.D., and Brinster, R.L. 1985. The c-*myc* oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature* **318,** 533-538.
3. Adams, J.M. 1988. Consequences of constitutional activation of oncogenes within transgenic mice. In Cellular Oncogene Activation, G. Klein, ed (New York: Marcel Dekker, Inc), pp. 365-388.
4. Alexander, W.S., Schrader, J.W., and Adams, J.M. 1987. Expression of the c-*myc* oncogene under control of an immunoglobulin enhancer in Eμ-*myc* transgenic mice. *Mol. Cell. Biol.* **7,** 1436-1444.
5. Alexander, W.S., Adams, J.M., and Cory, S. 1989. Oncogene cooperation in lymphocyte trans-formation: Malignant conversion of Eμ-myc trans-genic pre-B cells in vitro is enhanced by v-H-*ras* or v-*raf* but not *v-abl*. *Mol. Cell. Biol.* **9,** 67-73.
6. Alitalo, K., Ramsay, G., Bishop, J.M., Pfeifer-Ohlson, S., Colby, W.W., and Levinson, A.D. 1983a. Identification of nuclear proteins encoded by viral and cellular *myc* oncogenes. *Nature* **306,** 274-277.
7. Alitalo, K., Schwab, M., Lin, C.C., Varmus, H.E., and Bishop. J.M. 1983b. Homogeneously staining chromosomal regions contain amplified copies of an abundantly expressed cellular oncogene (c-*myc*) in malignant neuroendocrine cells from a human colon carcinoma. *Proc. Natl. Acad. Sci. USA* **80,** 1707-1711.
8. Alitalo, K., Winqvist, R., Keski-Oja, J., Ilvonen, M., Saksela, K., Alitalo, R., Laiho, M., Knuutila, S., and Chapelle, A. 1985. Acute myelogenous leukemia with c-*myc* amplification and double minute chromosomes. *Lancet* 1035-1039.
9. Alitalo, K. and Schwab, M., 1986. Oncogene amplification in tumor cells. *Adv. Cancer Res.* **47,** 235-281.
10. Amatruda, T.T., Sidell, N., Ranyard, J., and Koeffler, H.P. 1985. Retinoic acid treatment of human neuroblastoma cells is associated with decreased N-*myc* expression. *Biochem. Biophys. Res. Comm.*, **126,** 1189-1195.
11. Armelin, H.A., Armelin M.C.S., Kelly, K., Stewart, T., Leder, P., Cochran, B.H., and Stiles, C.D. 1984. Functional role for c-*myc* in mitogenic response to platelet-derived growth factor. *Nature* **310,** 655-660.
12. Asker, C., Mareni, C., Coviello, D., Ingvarsson, S., Sessarego, M., Origone, P., Klein, G., and Sümegi, J. 1988. Amplification of c-*myc* and DNA sequences homologous to murine *pvt* -1 region in human acute non lymphatic leukemia cells (ANLL,M1). *Leuk. Res.* **12,** 523-527.
13. Axelson, H., Pear, W.S., Bazin, H., Klein, G., and Sümegi, J. Deregulation of c-*myc* in IgH/*myc* (6;7) translocation carrying rat immunocytoma. Manuscript.
14. Banerjee, M., Wiener, F., Spira, J., Babonits, M., Nilsson, M.-G., Sümegi, J., and Klein, G. 1985. Mapping of the c-*myc*, *pvt* -1 and immunoglobulin kappa genes in relation to the mouse plasmacytoma-associated variant (6;15) translocation breakpoint. *EMBO J.* **4,** 3183-3188.
15. Bazin, H., Pear, W.S., and Sümegi, J. 1988. Louvain rat immunocytomas. *Adv. Cancer Res.* **50,** 279-310.
16. Beard, J.W. 1980. Biology of avian oncornaviruses. In "Viral Oncology" (G. Klein, ed) pp. 55-87. Raven Press, New York.
17. Bentley, D.L., and Groudine, M. 1986a. A block to elongation is largely responsible for decreased transcription of c-*myc* in differentiated HL60 cells. *Nature* **321,** 702-706.
18. Bentley, D.L., and Groudine, M. 1986b. Novel promoter upstream of the human c-*myc* gene and regulation of c-*myc* expression in B-cell lymphomas. *Mol. Cell. Biol.* **6,** 3481-3489.
19. Bernard, O., Cory, S., Gerondakis, S., Webb, E., and Adams, J.M. 1983. Sequence

of the murine and human cellular *myc* oncogenes and two modes of *myc* transcription resulting from chromosome translocation in B lymphoid tumors *EMBO J.* **2,** 2375-2383.

20. Birrer, M.J., Segal, S., DeGreve, J.S., Kaye, F., Sausville, E.A., and Minna, J.D. 1988. L-*myc* cooperates with *ras* to transform primary rat embryo fibroblasts. *Mol. Cell. Biol.* **8,** 2668-2673.
21. Birrer, M.J., Raveh, L., Dosaka, H., and Segal, S. 1989. A transfected L-*myc* gene can substitute for c-*myc* in blocking murine erythroleukemia differentiation. *Mol. Cell Biol.* **9,** 2734-2737.
22. Bishop, J.M. 1983. Cellular oncogenes and retro-viruses. *Annu. Rev. Biochem.* **52,** 301-354.
23. Bishop, J.M. 1987. The molecular genetics of cancer. *Science* **235,** 305-311.
24. Blanchard, J.-M., Piechaczyk, M., Dani, C., Chambard, J-C., Franchi, A., Pouyssegur, J., and Jeanteur, P. 1985. C-*myc* gene is transcribed at high rate in Go-arrested fibroblasts and is post-transcriptionally regulated in response to growth factors. *Nature* **317,** 443-445.
25. Bohman, D., Bos, T.J., Admon, A., Nishimura, T, Vogt, P.K., and Tjian, R. 1987. Human proto-oncogene c-*jun* encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. *Science* **238,**1386-1392.
26. Bonnieu, A., Piechaczyk, M., Marty, L., Cuny, M., Blanchard, J.M., Fort, P., and Jeanteur, P. 1988. Sequence determinats of c-*myc* turn-over: Influence of 3´ and 5´ non-coding regions. *Oncogene Res.* **3,** 155-166.
27. Bornkamm, G.E., Polack, A., and Eick, D. 1988. c-*myc* deregulation by chromosomal translocation in Burkitt´s lymphoma. In Cellular Oncogene Activation, G. Klein, ed. (New York: Marcel Dekker, Inc.), pp223-274.
28. Bos, J., Verlaan-deVries, M., Jansen, A., Veeneman, G., van Boom, J., and van der Eb, A. 1984. Three different mutations in codon 61 of the human N-*ras* gene detected by synthetic oligonucleotide hybridization. *Nucleic Acids Res.* **12,** 9155-9162.
29. Brewer, G. and Ross, J. 1988. Poly(A) shortening and degradation of the 3´ A+U-rich sequences of human c-*myc* mRNA in a cell-free system. *Mol. Cell. Biol.* **8,** 1697-1708.
30. Brewer, G. and Ross, J. 1989. Regulation of c-*myc* stability *in vitro* by a labile destabilizer with an essential nucleic acid component. *Mol. Cell. Biol.* **9,** 1996-2006.
31. Brodeur, G.M., Seeger, R.C., Schwab, M., Varmus, H.E., and Bishop, J.M. 1984. Amplification of N-*myc* in untreated human neuroblastomas correlates with advanced disease stage. *Science* **224,** 1121-1124.
32. Brooks, B.J., J. Battey, M.M. Neu, A.F. Gazdar, and J.D. Minna. 1986. Amplification and expression of the *myc* gene in small-cell lung cancer. *Adv. Viral Oncol.* **7,**155-171.
33. Campell, G.R., Zimmerman, K., Blank, R.D., Alt, F.W., and D´Eustachio. 1989. Chromosomal location of N-*myc* and L-*myc* genes in the mouse. *Oncogene Res.* **4,** 47-54.
34. Campisi, J., Gray, H.E., Pardee, A.B., Dean, M., and Sonensheim, G.E. 1984. Cell-cycle control of c-*myc* but not c-*ras* expression is lost following chemical transformation. *Cell* **36,** 241-247.
35. Cavalieri, F., and Goldfarb, M. 1987. Growth-factor-deprived BALB/c 3T3 murine fibroblasts can enter the S phase after induction of c-*myc* gene expression. *Mol. Cell. Biol.* **7,** 3554-3560.
36. Cavalieri, F., and Goldfarb, M. 1988. N-*myc* proto-oncogene expression can induce DNA replication in Balb/c 3T3 fibroblasts. *Oncogene* **2,** 289-291.
37. Cesarman, E., Dalla-Favera, R., Bentley, D., and Groudine, M. 1987. Mutations in the first exon are associated with altered transcription of c-*myc* in Burkitt lymphoma. *Science* **238,** 1272-1275.
38. Cheng, G. and Skoultchi, A.I. 1989. Rapid induction of polyadenylated H1 histone mRNA in mouse erythroleukemia cells is regulated by c-*myc*. *Mol. Cell. Biol.* **9,** 2332-2340.
39. Chiu, R., Boyle, W.J., Meek, J., Smeal, T., Hunter, T., and Karin, M. 1988. The c-*fos* interacts with c-*jun* /AP-1 to stimulate transcription of AP-1 responsive genes. *Cell* **54,** 541-552.
40. Chung, J., Sinn, E., Reed, R.R., and Leder, P. 1986. Trans-acting elements modulate expression of the human c-*myc* gene in Burkitt lymphoma cells. *Proc. Natl. Acad. Sci. USA* **83,** 7918-7922.
41. Classon, M., Henriksson, M., Sümegi, J., Klein, G., and Hammarskjöld M-L. 1987. Elevated c-*myc* expression facilitates the replication of SV40 DNA in human lymphoma cells. *Nature* **330,** 272-274.
42. Cleveland, J.L., Huleihel, M., Bressler, P., Siebenlist, U., Akiyama, L., Eisenman, R.N., and Rapp, U.R. 1988. Negative regulation of c-*myc* transcription involves *myc* family proteins. *Oncogene Res.* **3,** 357-375.

43. Clurman, B.E., and Hayward, W.S. 1988. Insertional activation of proto-oncogenes previoulsy identified as viral oncogenes. In Cellular Oncogene Activation, G. Klein, ed. (New York: Marcel Dekker, Inc.), pp. 55-94.
44. Collins, S., and Groudine, M. 1982. Amplification of endogenous *myc* -related DNA sequences in a human myeloid leukemia cell line. *Nature* **298,** 679-681.
45. Collins, S.J., and Groudine, M. 1988. Oncogene amplification and tumor progression. In *Cellular Oncogene Activation*, G. Klein, ed. (New York: Marcel Dekker, Inc.), pp. 313-334
46. Cole, M.D. 1986. The *myc* oncogene: its role in transformation and differentiation. *Ann. Rev. Genet.* **20,** 361-364.
47. Conscience, J.-F., Verrier, B., and Martin, G. 1986. Interleukin-3-dependent expression of the c-*myc* and c-*fos* proto-oncogenes in hemopoietic cell lines. *EMBO J.* **5,** 317-323.
48. Coppola, J. and Cole, M.D. 1986. Constitutive c-*myc* oncogene expression blocks mouse erythroleukemia cell differentiation but not commitment. *Nature* **320,** 760-763.
49. Corcoran, L.M., Adams, J.M., Dunn, A.R., and Cory, S. 1984. Murine T lymphomas in which the cellular *myc* oncogene has been activated by retroviral insertion. *Cell* **37,** 113-122.
50. Cory, S. 1986. Activation of cellular oncogenes in hemopoietic cells by chromosome translocation. *Adv. Cancer Res.* **47,** 189-234.
51. Coughlin, S.R., Lee, W.M.F., Williams, P.E., Giels, G.M., and Williams, L.T. 1985. c-*myc* gene expression is stimulated by agents that activate protein kinase C and does not account for the mitogenic effect of PDGF. *Cell* **43,** 243-251.
52. Cullen, B.R., Lomedico, P.T., and Ju, G. 1984. Transcriptional interference in avian retroviruses- implications for the promotor insertion model of leukaemogenesis. *Nature* **307,** 241-245.
53. Curran, T., Miller, A.D., Zokas, L., and Verma, I.M. 1984. Viral and cellular *fos* proteins: a comparative analysis. *Cell* **36,** 259-268.
54. Curran, T., and Morgan, J.I. 1985. Superinduction of c-*fos* by nerve growth factor in the presence of peripherally active benzodiazepines. *Science* **229,** 1265-1268.
55. Dalla-Favera, R., Gelmann, E.P., Martinotti, S., Franchini, G., Papas, T.S., Gallo, R.C., and Wong-Staal, F. 1982a. Cloning and characterization of different human sequences related to the *onc* gene (v-*myc*) of avian myelocytomatosis virus (MC29). *Proc. Natl. Acad. Sci. USA* **79,** 6497-6501.
56. Dalla-Favera, R., Wong-Stahl, F., and Gallo, R. 1982b. Oncogene amplification in promyelocytic leukemia cell line HL-60 and primary leukemic cells of the same patient. *Nature* **298,** 61-63.
57. Dalla-Favera, R., Bregni, M., Erikson, J., Patterson, D., Gallo, R.C., and Croce, C.M. 1982c. Human c-*myc onc* gene is located on the region of chromsosme 8 that is translocated in Burkitt lymphoma cells. *Proc. Natl. Acad. Sci. USA* **79,** 7824-7827.
58. Dang, C.V., and Lee, W.M.F. 1988. Identification of the human c-*myc* protein nuclear translocation signal. *Mol. Cell. Biol.* **8,** 4048-4054.
59. Dang, C.V., McGuire, M., Buckmire, M., and Lee, W.M.F. 1989. Involvement of the "leucine zipper" region in the oligomerization and transforming activity of human c-*myc* protein. *Nature* **337,** 664-666.
60. Dani, C., Blanchard, J.M., Piechaczyk, M., ElSabouty, S., Marty, L., and Jeanteur, P. 1984. Extreme instability of *myc* mRNA in normal and transformed human cells. *Proc. Natl. Acad. Sci. USA* **81,** 7046-7050.
61. Dani, C., Mechit, N., Piechaczyk, M., Lebleu, B., Jeanteur, P., and Blanchard, J.M. 1985. Increased rate of degradation of c-*myc* mRNA in interferon-treated Daudi cells. *Proc. Natl. Acad. Sci. USA* **82,** 4896-4899.
62. Dean, M., Levine, R., A., Ran, W., Kindy, M.S., Sonenshein, G.E., and Campisi, J. 1986a. Regulation of c-*myc* transcription and mRNA abundance by serum growth factors and cell contact. *J. Biol. Chem.*, **261,** 9161-9166.
63. Dean, M., Levine, R.A., and Campisi, J., 1986b. c-*myc* regulation during retinoic acid-induced differentiation of F9 cells is posttranscriptional and associated with growth arrest. *Mol. Cell. Biol,* **6,** 518-524.
64. Dean, M., Cleveland, J.L., Rapp, U.R., and Ihle, J.N. 1987. Role of *myc* in the abrogation of IL3 dependence of myeloid FDC-P1 cells. *Oncogene Res.* **1,** 279-296.
65. Dedieau, J.-F., Gazin, C., Rigolet, M., and Galibert, F.1988. Evolutionary conservation of the product of human c-*myc* exon 1 and its inducible expression in a murine cell line. *Oncogene* **3,** 523-529.
66. DeGreve, J., Battey, J., Fedorko, J., Birrer, M., Evan, G., Kaye, F., Sausville, E., and Minna, J. 1988. The human L-*myc* gene encodes multiple nuclear phosphoproteins

from alternatively processed mRNAs. *Mol. Cell. Biol.* **8,** 4381-4388.

67. DePinho, R.A., Legouy, E., Feldman, L.B., Kohl, N.E., Yancopoulos, G.D., and Alt F.W. 1986. Structure and expression of the murine N-*myc* gene. *Proc. Natl. Acad. Sci. USA* **83,** 1827-1831.
68. DePinho, R.A., Mitsock, L., Hatton, K., Ferrier, P., Zimmerman, K., Legouy, E., Tesfaye, A., Collum, R. Yancopoulos, G., Nisen, P., Kriz, R., and Alt, F.W. 1987a. *Myc* family of cellular oncogenes. *J. Cell. Biochem.* **33,** 257-266.
69. DePinho, R.A., Hatton, K.S., Tesfaye, A., Yancopoulos G.D., and Alt, F.W. 1987b. The human *myc* gene family; Structure and activity of L-*myc* and an L-*myc* pseudogene. *Genes Dev.* **1,** 1311-1326.
70. Dildrop, R., Ma, A., Zimmerman, K., Hsu, E., Tesfaye, A., DePinho, R., and Alt, F.W. 1989. IgH enhancer-mediated deregulation of N-*myc* gene expression in transgenic mice: generation of lymphoid neoplasias that lack c-*myc* expression. *EMBO J.* **8,** 1121-1128.
71. Distel, R.J., Ro, H.-S., Rosen, B.S., Groves, D.L., and Spiegelman, B.M. 1987. Nucleoprotein complexes that regulate gene expression in adipocyte differentiation: direct participation of c-*fos*. *Cell* **49,** 835-844.
72. Dmitrovsky, E., Kuehl, W., Hollis, G., Kirsch, I., Bender, T., and Segal, S. 1986. Expression of a transfected human c-*myc* oncogene inhibits differentiation of a mouse erythroleukemia cell line. *Nature* **322,** 748-750.
73. Donner, P., Greiser-Wilke, I., and Moelling, K. 1982. Nuclear localization and DNA binding of the transforming gene product of avian myelocytomatosis virus. *Nature* **296,** 262-266.
74. Dony, C., Kessel, M., and Gruss, P. 1985. Post-transcriptional control of *myc* and p53 expression during differentiation of the embryonal cell line F9. *Nature* **317,** 636-639.
75. Eick, D., Piechaczyk, M., Hegnlein, B., Blanchard, J.-M., Traub, B., Kofler, E., Wiest, S., Lenoir, G.M., and Bornkamm, G.W. 1985. Aberrant c-*myc* RNAs of Burkitt´s lymphoma cells have longer half-lives. *EMBO J.* **4,** 3717-3725.
76. Eick, D., and Bornkamm, G.W. 1986. Transcriptional arrest within the first exon in a fast control mechanism in c-*myc* gene expression. *Nucleic Acids Res.* **14,** 8331-8346.
77. Eick, D., Polack, A., Kofler, E., and Bornkamm, G.W. 1988. The block of elongation in c-*myc* exon 1 is abolished in Burkitt´s lymphoma cell lines with variant translocation. *Oncogene* **3,** 397-403.
78. Einat, M., Resnitzky, D., and Kimchi, A. 1985. Close link between reduction of c-*myc* expression by interferon and Go/G1 arrest. *Nature* **313,** 597-600.
79. Eisenman, R.N., and Thompson, C.B. 1986. Oncogenes with potential nuclear function: *myc*, *myb* and *fos*. *Cancer Surv.* **5,** 309-327.
80. Erikson, J., Miller, D.A., Miller, O.J., Abcarian, P.W., Skurla, R.M., Mushinski, J.F., and Croce, C.M. 1985. The c-*myc* oncogene is translocated to the involved chromosome 12 in mouse plasmacytoma. *Proc. Natl. Acad. Sci. USA* **82,** 4212-4216.
81. Escot, C., Theillet, C., Lidereau, R., Spyratos, F., Champeme, M.H., Gest, J., and Callahan, R. 1986. Genetic alteration of the c-*myc* protooncogene (*MYC*) in human primary breast carcinomas. *Proc. Natl. Acad. Sci. USA* **83,** 4834-4838.
82. Evan, G.I., Hancock, D.C., Littlewood, T.D., and Gee, N.S. 1986. Characterization of human *myc* proteins. *Curr. Top. Microbiol. Immunol.* **132,** 362-374.
83. Evan, G.I., Moore, J.P., Ibson, J.M., Waters, C.M., Hancock, D.C., and Littlewood, T.D. 1988. Immunological probes in the analysis of *myc* protein expression. *Curr. Top. Microbiol. Immunol.* **141,** 189-201.
84. Evan, G.I., Ibson, J., Waters, C., Hancock, D., and Littlewood, T.D. *Mol. Cell. Biol.* **9,** in press.
85. Fahrlander, P.D., Sümegi, J., Yang, J.-Q., Wiener, F., Marcu, K.B., and Klein, G. 1985. Activation of the c-*myc* oncogene by the immunoglobulin heavy-chain gene enhancer after multiple switch-region-mediated chromosome rearrangements in a murine plasmacytoma. *Proc. Natl. Acad. Sci. USA* **82,** 3746-3750.
86. Ferré, F., Martin, P. Lagrou, C., Raes, M.-B., Ghysdael, J., Saule, S., and Stéhelin, D. 1987. The human c-*myc* exon 1 product: preparation of antisera and analysis of its expression. *Oncogene* **1,** 387-393.
87. Figge, J., Webster, T., Smith, T.F., and Paucha E. 1988. Prediction of similar transforming regions in simian virus 40 large T, adenovirus E1A, and *myc* oncoproteins. *J. Virol.* **62,** 1814-1818.
88. Finger, L.R., Huebner, K., Cannizzaro, L.A., McLeod, K., Nowell, P.C., and Croce, C.M. 1988. Chromosomal translocation in T-cell leukemia line HUT78 results in a *MYC*

fusion transcript. *Proc. Natl. Acad. Sci. USA* **85,** 9158-9162.

89. Fink, K.L., Wieben, E.D., Woloschak, G.E., and Spelsberg, T.C. 1988. Rapid regulation of c-*myc* protooncogene expression by progesterone in the avian oviduct. *Proc. Natl. Acad. Sci. USA* **85,** 1796-1800.
90. Floyd-Smith, G., Slattery, E., and Lengyel, P. 1981. Interferon action: RNA cleavage pattern of a (2´-5´) oligoadenylate-dependent endonuclease. *Science* **212,** 1030-1032.
91. Foulds, L. 1958. The natural history of cancer. *J. Chron. Dis.* **8,** 2-37.
92. Freytag, S.O. 1988. Enforced expression of the c-*myc* oncogene inhibits cell differentiation by precluding entry into a distinct predifferentiation stage in Go/G1. *Mol. Cell. Biol.* **8,** 1614-1624.
93. Gariglio, P., Angel, R., Herrera, A., and Bonilla, M. 1987. Theoretical model for the post-transcriptional regulation of the human c-*myc* gene expression, involving double-stranded RNA processing. *J. Theor. Biol.* **125,** 83-92.
94. Gazin, C., deDinechin, Dupont, S., Hampe, A., Masson, J.M., Martin, P., Stehelin, D., and Galibert, F. 1984. Nucleotide sequence of the human c-*myc* locus: Provocative open reading frame within the first exon. *EMBO J.* **3,** 383-387.
95. Gazin, C., Rigolet, M., Briand, J.P., VanRegenmortel, M.H.V., and Galibert, F. 1986. Immunochemical detection of proteins related to the human c-*myc* exon 1. *EMBO J.* **5,** 2241-2250.
96. Goyette, M., Petropoulos, C.J., Shank, P.R., and Fausto, N. 1984. Regulated transcription of c-Ki-*ras* and c-*myc* during compensatory growth of rat liver. *Mol. Cell. Biol.* **4,** 1493-1498.
97. Graham, M., Adams, J.M., and Cory, S. 1985. Murine T lymphomas with retroviral inserts in the chromosome 15 locus for plasmacytoma variant (6;15) translocations. *Nature* **314,** 740-743.
98. Graham, M., and Adams, J.M. 1986. Chromosome 8 breakpoint far 3´ of the c-*myc* oncogene in a Burkitt´s lymphoma 2;8 variant translocation is equivalent to the murine *pvt*-1 locus. *EMBO J.* **5,** 2845-2851.
99. Greenberg, M.E. and Ziff, E.B. 1984. Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. *Nature* **311,** 433-438.
100. Greenberg, M.E., Greene, L.A., and Ziff, E.B. 1986. Nerve growth factor and epidermal growth factor induce rapid transient changes in proto-oncogene tanscription in PC12 cells. *J. Biol. Chem.* **260,** 14101-14110.
101. Griep, A.E. and DeLuca, H.F. 1986. Decreased c-*myc* expression is an early event in retinoic acid induced differentiation of F9 teratocarcinoma cells. *Proc. Natl. Acad. Sci. USA.* **83,** 5539-5543.
102. Griep, A.E., and Westphal, H. 1988. Antisense *Myc* sequences induce differentiation of F9 cells. *Proc. Natl. Acad. Sci. USA.* **85,** 6806-6810.
103. Guérin M., Barrois, M., Terrier, M.J., Spielmann, M., and Riou, G. 1988. Overexpression of either c-*myc* of c-*erb* B-2/*neu* proto-oncogenes in human breast carcinomas: Correlation with poor prognosis. *Oncogene Res.* **3,** 21-31.
104. Haluska, F.G., Finver, S., Tsujimoto, Y., and Croce, C.M. 1986. The t(8;14) chromosomal translocation occurring in B-cell malignancies results from mistakes in V-D-J joining. *Nature* **324,** 158-161.
105. Hammerling, U., Bjelfman, C., and Påhlman, S. 1987. Different regulation of N-*myc* and c-*myc* expression during phorbol ester-induced maturation of human SH-SY5Y neuroblastoma cells. *Oncogene* **2,** 73-77.
106. Hann, S. R., and Eisenman, R.N. 1984. Proteins encoded by the human c-*myc* oncogene: Differential expression in neoplastic cells. *Mol. Cell. Biol,* **4,** 2486-2497.
107. Hann, S.R., Thompson, C.B., and Eisenman, R.N. 1985. c-*myc* oncogene protein synthesis is independent of the cell cycle in human and avian cells. *Nature* **314,** 366-369.
108. Hann, S.R., King, M.W., Bentley, D.L., Anderson, C.W., and Eisenman, R.N. 1988. A non-AUG translation initiation in c-*myc* exon 1 generates an N-terminally distinct protein whose synthesis is disrupted in Burkitt´s lymphomas. *Cell* **52,** 185-195.
109. Hay, N. and Aloni, Y. 1985. Attenuation of late simian virus 40 mRNA synthesis is enhanced by the agnoprotein and is temporally regulated in isolated nuclear systems. *Mol. Cell. Biol.* **5,** 1327-1334.
110. Hay, N., Takimoto, M., and Bishop, J.M. 1989. A *FOS* protein is present in a complex that binds a negative regulator of *MYC*. *Genes Dev.* **3,** 293-303.
111. Hayashi, K., Makino, R., Kawamura, H., Arisawal, A, and Yoneda, K. 1987. Characterization of rat c-*myc* and adjacent regions. *Nucl. Acids Res.* **15,** 6419-6436.
112. Hayata, I., Seki, M., Yoshida, K. Hirashima, K., Sado, T., Yamogowa, J.,

and Ishihara, T. 1983. Chromosomal aberrations observed in 52 mouse myeloid leukemias. *Cancer Res.* **43,** 367-373.

113. Hayday, A.D., Gillies, S.D., Saito, H., Wood, C., Wiman, K., Hayward, W.S., and Tonegawa S. 1984. Activation of a translocated human c-*myc* gene by an enhancer in the immunoglobulin heavy-chain locus. *Nature* **307,** 334-340.
114. Hayward, W.G., B.E. Neel, and S.M. Astrin. 1981. Activation of a cellular *onc* gene by promoter insertion in ALV-induced lymphoid leukosis. *Nature* **290,** 475-480.
115. Henglein, B., Synovzik, H., Groitl, P., Bornkamm, G.W., Hartl, P., and Lipp, M. 1989. Three breakpoints of variant t(2;8) translocations in Burkitt's lymphoma cells fall within a region 140 kilobases distal from c-*myc*. *Mol. Cell. Biol.* **9,** 2105-2113.
116. Hirai, S.-I., Ryseck, R.-P., Mechata, F., Bravo, R., and Yaniv, M. 1989. Characterization of *jun* D: a new member of the *jun* proto-oncogene family. *EMBO J.* 8, 1433-1439.
117. Horii, Y., Sugimoto, T., Sawada T., Imanishi, J., Tsuboi, K., and Hatanaka, M. 1989. Differential expression of N-*myc* and c-*src* proto-oncogenes during neuronal and Schwannian differentiation of human neuroblastoma cells. *Int. J. Cancer* **43,** 305-309.
118. Iguchi-Ariga, S.M.M., Itani, T., Kiji, Y., and Ariga, H. 1987. Possible function of the c-*myc* product: promotion of cellular DNA replication. *EMBO J.* **6,** 2365-2371.
119. Iguchi-Ariga, S., Okazaki, T., Itani, T., Ogata, M., Sato, Y., and Ariga, H. 1988. An initiation site of DNA replication with transcriptional enhancer activity present upstream of the c-*myc* gene. *EMBO J.* **7,** 3135-3142.
120. Ikegaki, N., Minna, J., and Kennett, R.H. 1989. The human L*myc* gene is expressed as two forms of protein in small cell lung carcinoma cell lines: detection by monoclonal antibodies specific to two *myc* homology box sequences. *EMBO J.* **8,** 1793-1799
121. Jakobovits, A., Schwab, M., Bishop, J.M., and Martin, G.R. 1985. Expression of N-*myc* in teratocarcinoma stem cells and mouse embryos. *Nature* **318,** 188-191.
122. Jolicoeur, P., Villeneuve, L., Rassart, E., and Kozak, C. 1985. Mouse chromosomal mapping of a murine leukemia virus integration region (*Mis* -1) first identified in rat thymic leukemia. *J. Virol.* **56,** 1045-1048.
123. Jones, T, and Cole, M. 1987. Rapid cytoplasmic turnover of c-*myc* mRNA: Requirement of the 3′ untranslated sequences. *Mol. Cell. Biol.* **7,** 4513-4521.
124. Kaczmarek, L., Hyland, J.K., Watt, R., Rosenberg, M., and Baserga, R. 1985. Microinjected c-*myc* as a competence factor. *Science* **228,** 1313-1315.
125. Kaddurah-Daouk, R., Greene, J.M., Baldwin. A.S., and Kingston, R.E. 1987. Activation and repression of mammalian gene expression by the c-*myc* protein. *Gen. Dev.* **1,** 347-357.
126. Katz, A. and Kahana, C. 1989. Rearrangement between ornithine decarboxylase and the switch region of the γ1immunoglobulin gene in α-difluoromethyl-ornithine resistant mouse myeloma cells. *EMBO J.* **8,** 1163-1167.
127. Kaye, F., Battey, J., Nau, M., Brooks, B., Seifter, E., DeGreve, J., Birrer, M., Sausville, E., and Minna, J. 1988. Structure and expression of the human L-*myc* gene reveals a complex pattern of alternative mRNA processing. *Mol. Cell. Biol.* **8,** 186-195.
128. Keath, E.J., Caimi, P.G., and Cole, M.D. 1984a. Fibroblast lines expressing activated c-*myc* oncogenes are tumorigenic in nude mice and syngeneic animals. *Cell* **39,** 339-348.
129. Keath, E.,J., Kelekar, A., and Cole, M.D. 1984b. Transcriptional activation of the translocated c-*myc* oncogene in mouse plasmacytomas: Similar RNA levels in tumor and proliferating normal cells. *Cell* **37,** 521-528.
130. Kelekar, A. and Cole, M.D. 1986. Tumorigenicity of fibroblast lines expressing the adenovirus E1A, cellular p53, and normal c-*myc* genes. *Mol. Cell. Biol.* **6,** 7-14.
131. Kelly, K., Cochran, B.H., Stiles, C.D., and Leder, P. 1983. Cell-specific regulation of the c-*myc* gene by lymphocyte mitogenes and platelet-derived growth factor. *Cell* **35,** 603-610.
132. Kerppola, T.K., and Kane, C.M. 1988. Intrinsic sites of transcription termination and pausing in the c-*myc* gene. *Mol. Cell. Biol.* **8,** 4389-4394.
133. Kingston, R.E., Baldwin A.S., and Sharp, P.A. 1984. Regulation of heat shock protein 70 gene expression by c-*myc*. *Nature* **312,** 280-282.
134. Klein, G. and Klein, E. 1985. *Myc* /Ig juxtaposition by chromosmal translocations: some new insights, puzzles and paradoxes. *Immunol. Today* **6,** 208-215.

135. Klein, G. 1988. Oncogenes and suppressor genes. *Acta Oncol.* **27,** 427-437.
136. Klein, S., Sablitzky, F., and Radbruch, A. 1984. Deletion of the Ig enhancer does not reduce immunoglobulin heavy chain production of a hybridome IgD class switch variant. *EMBO J.* **3,** 2473-2476.
137. Klempnauer, K.-H., Symonds, G., Evan, G.I., and Bishop, J.M. 1984. Subcellular localization of proteins encoded by oncogenes of avian myeloblastosis virus and avian leukemia virus E26 and the chicken c-*myb* gene. *Cell* **37,** 537-547.
138. Koehne, C.F., Lazo, P.A., Alves, K., Lee, J.S., Tsichlis, P.N., and O'Donnell, P.V. 1989. The *Mlvi* -1 locus involved in the induction of rat T-cell lymphomas and the *pvt* -1/*Mis* -1 locus are identical. *J. Virol.* **63,** 2366-2369.
139. Kohl, N.E., Kanda, N., Schreck, R.R., Bruns, G., Latt, S.A., Gilbert, F., and Alt. F.W. 1983. Transposition and amplification of oncogene-related sequences in human neuroblastomas. *Cell* **35,** 359-367.
140. Kohl, N.E., Legouy, E., DePinho, R.A., Nisen, P.D., Smith, R.K., Gee, C.E., and Alt, F.W. 1986. Human N-*myc* is closely related in organization and nucleotide sequence to c-*myc*. *Nature* **319,** 73-77.
141. Kozbor, D., and Croce, C.M. 1984. Amplification of the c-*myc* oncogene in one of five human breast carcinoma cell lines. *Cancer Res.* **44,** 438-441.
142. Krystal, G., Birrer, M., Way, J., Nau, M., Sausville E., Thompson, C., Minna, J., and Battey J. Multiple mechanisms for transcriptional regulation of the *myc* gene family in small-cell lung cancer. 1988. *Mol. Cell. Biol.* **8,** 3373-3381.
143. Lachman, H.M., and Skoultchi, A.I., 1984. Expression of c-*myc* changes during differentiation of mouse erythroleukemia cells. *Nature* **310,** 592-594.
144. Lacy, J., Sarkar, S.N., and Summers, W.C. 1986. Induction of c-*myc* expression in human B lympocytes by B-cell growth factor and anti-immunoglobulin. *Proc. Natl. Acad. Sci. USA* **83,** 1458-1462.
145. Land, H., Parada, L.F., and Weinberg, R.A. 1983. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature* **304,** 596-601.
146. Land, H., Chen, A.C., Morgenstern, J.P., Parada, L.F., and Weinberg, R.A. 1986. Behaviour of *myc* and *ras* oncogenes in tranformation of rat embryo fibroblasts. *Mol. Cell. Biol.* **6,** 1917-1925.
147. Landschulz, W.H., Johnson, P.F., and McKnight, S.L. 1988. The Leucine Zipper: A hypothetical structure common to a new class of DNA binding proteins. *Science* **240,** 1759-1764.
148. Lautenberger, J.A., Schulz, R.A., Garon C.F., Tsichlis, P.N., and Papas, T.S. 1981. Molecular cloning of avian myelocytomatosis virus (MC29) transforming sequences. *Proc. Natl. Acad. Sci. USA* **78,** 1518-1522.
149. Lavialle, C., Modjtahedi, N., Cassingena, R., and Lhomme, C. 1988. High c-*myc* amplification level contributes to the tumorigenic phenotype of the human breast carcinoma cell line SW 613-S. *Oncogene* **3,** 335-339.
150. Leder, A. Pattengale, P.K., Kuo, A., Stewart, T.A., and Leder, P. 1986. Consequences of widespread deregulation of the c-*myc* gene in transgenic mice: multiple neoplasms and normal development. *Cell* **45,** 485-495.
151. Legouy, E., DePinho, R., Zimmerman, K., Collum R., Yancopoulos, G., Mitsock, L., Kriz, R., and Alt, F.W. 1987. Structure and expression of the murine L-*myc* gene. *EMBO J.* **6,** 3359-3366.
152. Lemay, G., and Jolicoeur, P. 1984. Rearrangement of a DNA sequence homologous to a cell-virus junction fragment in several Moloney murine leukemina virus-induced rat thymomas. *Proc. Natl. Acad. Sci. USA.* **81,** 38-42.
153. Levan, A., Levan, G., and Mandahl, N. 1981. in *Genes, Chromosomes and Neoplasia* (eds. Arrghi, F.E., Rao, P.N. and Stubblefield, E.) pp223-251 (Raven; New York).
154. Li, Y., Holland, D.A., harley, J.W., and Hopkins, N. 1984. Viral integration near c-*myc* in 10-20% of MCF 247-induced AKR lymphomas. *Proc. Natl. Acad. Sci. USA.* **81,** 6808-6811.
155. Liboi, E., Pelosi, E., Testa, U., Peschle, C., and Rossi, G.B. 1986. Proliferative response and oncogene expression induced by epidermal growth factor in EL2 rat fibroblasts. *Mol. Cell. Biol.* **6,** 2275-2278.
156. Linial, M., and Groudine, M. 1985. Transcription of three c-*myc* exons is enhanced in chicken bursal lymphoma cell lines. *Proc. Natl. Acad. Sci. USA* **82,** 53-57.
157. Lipp, M., Schilling, R., Wiest, S., Laux, G., and Bornkamm, G.W. 1987. Target sequences for *cis* -acting regulation within the dual promoter of the human c-*myc* gene. *Mol. Cell. Biol.* **7,** 1393-1400.

158. Little, C.D., M.M. Nau, D.N. Carney, A.F. Gazdar, and J.D. Minna. 1983. Amplification and expression of the c-*myc* oncogene in human lung cancer cell lines. *Nature* **306,** 194-196.
159. Lüscher, B., and Eisenman, R.N. 1988. c-*myc* and c-*myb* protein degradation: Effect of metabolic inhibitors and heat shock. *Mol. Cell. Biol.* **8,** 2504-2512.
160. Lüscher, B., Kuenzel, E.A., Krebs, E.G., and Eisenman, R.N. 1989. *Myc* oncoproteins are phosphorylated by casein kinase II. *EMBO J.* **8,** 1111-1119.
161. MacAuley, A., and Pawson, T. 1988. Cooperative transforming activites of *ras*, *myc*, and *src* viral oncogenes in non-established rat adrenocortical cells. *J. Virol.* **62,** 4712-4721.
162. Madhavan, K., Bilodeau-Wentworth, D., and Wadsworth, S.C. 1985. Family of developmentally regulated, maternally expressed Drosophila RNA species detected by a v-*myc* probe. *Mol. Cell. Biol.* **5,** 7-16.
163. Magrath, I. 1989. The pathogenesis of Burkitt's lymphoma. *Adv. Cancer Res.* in press.
164. Mäkelä, T.P., Saksela, K., and Alitalo, K. 1989. Two N-*myc* polypeptides with distinct amino termini encoded by the second and third exons of the gene. in press.
165. Makino, R., Hayashi, K., and Sugimura, T. 1984. C-*myc* transcript is induced in rat liver at a very early stage of regeneration or by cycloheximide treatment. *Nature* **310,** 697-698.
166. Marcu, K.B., Harris, L.J., Stanton, L.W., Erikson, J., Watt, R., and Croce, C.M. 1983. Transcriptionally active c-*myc* oncogene is contained within NIARD, a DNA sequence associated with chromosome translocations in B-cell neoplasia. *Proc. Natl. Acad. Sci. USA* **80,** 519-523.
167. Martinsson, T., Ståhl, F., Pollwein, P., Wenzel, A., Levan, A., Schwab, M., and Levan G. 1988. Tumorigenicity of SEWA murine cells correlates with degree of c-*myc* amplification. *Oncogene* **3,** 437-441.
168. Mehmet, HJ., Sinnett-Smith, J., Mooore, J.P., Evan, G.I., and Rozengurt, E. 1988. Differential induction of c-*fos* and c-*myc* by cyclic AMP in Swiss 3T3 Cells: Significance for the mitogenic response. *Oncogene Res.* **3,** 281-286.
169. Minarovits, J., Steinitz, M., Boldog, F., Imreh, I., Wirschubsky, Z., Ingvarsson, S., Hedenskog, M., Kormuta, S.M., and Klein, G. Differences in c-*myc* and *pvt* - 1 amplification in SEWA sarcoma sublines selected for adherent or non-adherent growth. Manuscript.
170. Mitani, K., Kurosawa, H., Suzuki,A., Hayashi, Y., Hanada, R., Yamamoto, K., Komatsu, A., Kobayashi, N., Nakagome, Y., and Yamada, M. 1986. Amplification of N-*myc* in a rhabdomyosarcoma. *Jpn. J. Cancer Res. (GANN)* **77,** 1062-1065.
171. Mitchell, R.L., Zokas, L., Schreiber, R.D., and Verma I.M. 1985. Rapid induction of the expression of proto-oncogene *fos* during human monocytic differentiation. *Cell* **40,** 209-217.
172. Morse, B. South, V.J., Rothberg, P.G., and Astrin, S.M. 1989. Somatic mutation and transcriptional deregulation of *myc* in endemic Burkitt's lymphoma disease: Heptamer-nonamer recognition mistakes? *Mol. Cell. Biol.* **9,** 74-82.
173. Mougneau, E., Lemieux, L., Rassoulzadegan, M., and Cuzin, F. 1984. Biological activities of v-*myc* and rearranged c-*myc* oncogenes in rat fibroblast cells in culture. *Proc. Natl. Acad. Sci. USA* **81,** 5758-5762.
174. Mugrauer, G., Alt, F.W., and Ekblom, P. 1988. N-*myc* proto-oncogene expression during organogenesis in the developing mouse as revealed by *in situ* hybridization. *J. Cell. Biol.* **107,** 1325-1335.
175. Müller, R., Klempnauer, K.-H., Moelling, K., Beug, H., and Vennström, B. 1988. Cellular oncogenes encoding nuclear proteins: *fos, myb, myc,* and *erb* A. In Cellular Oncogene Activation, G. Klein, ed (New York: Marcel Dekker, Inc), pp. 1-54.
176. Murray M., Cunningham, J., Parada, L., Dautry, F., Lebowithz, P., and Weinberg, R. 1983. The HL-60 transforming sequence: A *ras* oncogene cooexisting with altered *myc* genes in hematopoietic tumors. *Cell* **33,** 749-758.
177. Murre, C., McCaw, P.S., and Baltimore, D. 1989. A new DNA binding and dimerization motif in immunoglobulin enhancer binding, *daughterless*, *MyoD*, and *myc* proteins. *Cell* **56,** 777-783.
178. Mushinski, J.F. 1988. c-*myc* oncogene activation and chromosomal translocation in BALB/c plasmacytomas. In Cellular Oncogene Activation, G. Klein, ed. (New York: Marcel Dekker, Inc.), pp. 181-222.
179. Nau, M.M., Brooks, G.J. Battey, J., Sausville, E., Gazdar, A.F., Kirsch, I.R., McBride, O.W., Bertness, V., Hollis, G.F., and Minna, J.D. 1985. L-*myc*, a new *myc* - related gene amplified and expressed in

human small cell lung cancer. *Nature* **318,** 69-73.

180. Nepveu, A., and Marcu, K.B. 1986. Intragenic pausing and anti-sense transcription within the murine c-*myc* locus. *EMBO J.* **5,** 2859-2865.

181. Nisen, P.D., Zimmerman, K.A., Cotter, S.V., Gilbert, F, and Alt, F.W. 1986. Enhanced expression of the N-*myc* gene in Wilm´s tumors. *Cancer Res.* **46,** 6217-6222.

182. Norris, M.D., Brian, M.J., Vowels, M.R., and Stewart, B.W. 1988. N-*myc* amplification in Wilms´ tumor. *Cancer Genet. Cytogenet.* **30,** 187-189.

183. Nusse, R. 1986. The activation of cellular oncogenes by retroviral insertion. *Trends in Gen.* **3,** 244-247.

184. Nusse, R., and Berns, A. 1988. Cellular oncogene activation by insertion of retroviral DNA. Genes identified by provirus tagging. In *Cellular Oncogene Activation*, G. Klein, ed. (New York: Marcel Dekker, Inc), pp. 95-119.

185. O´Donnel, P.V., Fleissner, E., Lonial,. H., Koehne, C.F., and Reicin, A. 1985. Early clonality and high-frequency proviral integration into the c-*myc* locus in AKR leukemias. *J. Virol.* **55,** 500-503.

186. Ohno, S., Babonits, M., Wiener, F., Spira, J., Klein, G., and Potter, M. 1979. Nonrandom chromosome changes involving the Ig gene-carrying chromsomes 12 and 6 in pristane-induced mouse plasmacytomas. *Cell* **18,** 1001-107.

187. Ohno, S., Migita, S., Wiener, F., Babonits, M., Klein, G., Mushinski, J.F., and Potter, M. 1984. Chromosomal translocations activating *myc* sequences and transduction of v-*abl* are critical events in the rapid induction of plasmacytomas by pristane and Abelson virus. *J. Exp. Med.* **159,** 1762-1777.

188. Onclercq, R., Gilardi, P., Lavenu, A., and Cremisi, C. 1988. c-*myc* products trans-activate the adenovirus E4 promoter in EC stem cells by using the same target sequence as E1A products. *J. Virol.* **62,** 4533-4537.

189. Onclercq, R., Babinet, C., and Cremisi, C. 1989. Exogenous c-*myc* gene overexpression interferes with early events in F9 cell differentiation. *Oncogene Res.* **4,** 293-302.

190. Oren, M., Maltzman, W., and Levine, A.J. 1981. Post-translational regulation of the 54K cellular tumor antigene in normal and transformed cells. *Mol. Cell. Biol.* **1,** 101-110.

191. Payne, , G.S., Bishop, J.M., and Varmus, H. E. 1982. Multiple arrangements of viral DNA and an activated host oncogene in bursal lymphomas. *Nature* **295,** 209-217.

192. Pear, W.S., G. Wahlström, S. Nelson, S. Ingvarsson, H. Bazin, G. Klein, and J. Sümegi. 1986a. C-*myc* activation in spontaneously arising rat immunocytomas containing a 6:7 translocation: *Curr. Top. Microbiol. Immunol.* **132,** 169-174.

193. Pear, W.S., Wahlström, G., Szpirer, J., Levan, G., Klein, G., and Sümegi, J. 1986b. Localization of the rat immunoglobulin heavy chain locus to chromsome 6. *Immunogenetics* **23,** 393-395.

194. Pear, W.S., Wahlström, G., Nelson, S.F., Axelson, H., Szeles, A., Wiener, F., Bazin, H., Klein, G., and Sümegi, J. 1988a. 6;7 chromosomal translocation in spontaneously arising rat immunocytoams: Evidence for c-*myc* breakpoint clustering and correlation between isotypic expression and the c-*myc* target. *Mol. Cell. Biol.* **8,** 441-451.

195. Pear, W.S., Nelson, S.F., Axelson, H., Wahlström, G., Bazin, H., Klein, G., and Sümegi, J. 1988b. Aberrant class switching juxtaposes c-*myc* with a middle repetitive element (LINE) and an IgH intron in two spontaneously arising rat immunocytomas. *Oncogene* **2,** 499-507.

196. Pegg, A.E., and McCann, P.P. 1982. Polyamine metabolism and function. *Am. J. Physiol.* **243,** 212-221.

197. Pei, R. and Calame, K. 1988. Differential stability of c-*myc* mRNAS in a cell-free system. *Mol. Cell. Biol.* **8,** 2860-2868.

198. Pelicci, P.-G., Knowles, D.M., Magrath, I., and Dalla-Favera, R. 1986. Chromosomal breakpoints and structural alterations of the c-*myc* locus differ in endemic and sporadic forms of Burkitt lymphoma. *Proc. Natl. Acad. Sci. USA* **83,** 2984-2988.

199. Perlman, C., Sümegi, J., Szpirer, J., Levan, G., and Klein, G. 1985. The rat immunoglobulin kappa light chain locus is on chromosome 4. *Immunogenetics* **22,** 97-100.

200. Persson, H., and Leder, P. 1984a. Nuclear localization and DNA binding properties of a protein expressed by human c-*myc* oncogene. *Science* **225,** 718-721.

201. Persson, H. Hennighausen, L., Taub, R., DeGrado, W., and Leder, P. 1984b. Antibodies to human c-*myc* oncogene product: Evidence of an evolutionary conserved protein induced during cell proliferation. *Science* **225,** 687-693.

202. Persson, H., Gray, H.E., and Godeau, F. 1985. Growth-dependent synthesis of c-*myc*

-encoded proteins: Early stimulation by serum factors in synchronized mouse 3T3 cells. *Mol. Cell. Biol.* **5,** 2903-2912.

203. Piechaczyk, M., Yang, J.-Q., Blanchard, J.-M., Jeanteur, P., and Marcu, K.B. 1985. Post-transcriptional mechanism are responsible for accumulation of truncated c-*myc* RNAs in murine plasma cell tumors. *Cell* **42,** 589-597.
204. Pochedly, C 1982. Neuroblastoma. Elsevier, Bio-medical Press, New York.
205. Potter, M., Mushinski, J.F., Mushinski, E.B., Brust, S., Wax, J.S., Wiener, F., Babonits, M., Rapp, U.R., and Morse, H.C. 1987. Avian v-*myc* replaces chromosomal translocation in murine plasmacytoma-genesis. *Science* **235,** 787-789.
206. Prendergast, G.C., and Cole, M.D. 1989. Post-transcriptional regulation of cellular gene expression by the c-*myc* oncogene. *Mol. Cell. Biol.* **9,** 124-134.
207. Prochownik, E. and Kukowska, J. 1986. Deregulated expression of c-*myc* by murine erythroleukemia cells prevents differentiation. *Nature* **322,** 848-850.
208. Prochownik, E.V., Kukowska, J., and Rodgers, C. 1988. c-*myc* antisense transcripts accelerate differentiation and inhibit G_1 progression in murine erythroleukemia cells. *Mol. Cell. Biol.* **8,** 3683-3695.
209. Rabbits, P.H., Forster, A., Stinson, M.A., and Rabbits, T.H. 1985. Truncation of exon 1 from the c-*myc* gene results in prolonged c-*myc* mRNA stability. *EMBO J.* **4,** 3727-3733.
210. Ramsay, T., Stanton, L., Schwab, M., and Bishop, J.M. 1986. Human proto-oncogene N-*myc* encodes nuclear proteins that bind DNA. *Mol. Cell. Biol.* **6,** 4450-4457.
211. Ran W., Dean, M., Levine, R.A., Henkle, C., and Campisi, J. 1986. Induction of c-*fos* and c-*myc* mRNA by epidermal growth factor or calcium ionophore is cAMP dependent. *Proc. Natl. Acad. Sci. USA* **83,** 8216-8220.
212. Rechsteiner, M., Rogers, S., and Rote, K. 1987. Protein structure and intracellular stability. *TIBS* **12,** 390-394.
213. Reed, J.C., Nowell, P.C., and Hoover, R.G. 1985a. Regulation of c-*myc* mRNA levels in normal human lymphocytes by modulators of cell proliferation. *Proc. Natl. Acad. Sci. USA* **82,** 4221-4224.
214. Reed, J.C., Sabath,, D.E., Hoover, R.G., and Prystowsky, M.B. 1985b. Recombinant interleukin 2 regulates levels of c-*myc* mRNA in a clonal murine T lymphocyte. *Mol. Cell. Biol.* **5,** 3361-3368.
215. Reed, J.C., Alpers, J.D., Nowell, P.C., and Hoover, R.G. 1986. Sequential expression of proto-oncogene during lectin-stimulated mitogenesis of normal human lymphocytes. *Proc. Natl. Acad. Sci. USA* **83,** 3982-3986.
216. Reich, N.C., and Levine, A.J. 1984. Growth regulation of a cellular tumor antigen, p53, in nontransformed cells. *Nature* **308,** 199-201.
217. Reitsma, P.H., Rothberg, P.G., Astrin, S.,M., Trial, J., Bar-Shavit, Z., Hall, A., Teitelbaum, S.L., and Kahn, A.J. 1983. Regulation of *myc* gene expression in HL60 leukemia cells by a vitamin D metabolite. *Nature* **306,** 492-494.
218. Remmers, E.F., Yang, J.Q., and Marcu, K.B. 1986. A negative transcriptional control element resides upstream of the murine c-*myc* gene. *EMBO J.* **5,** 899-904.
219. Resnitzky, D., Yardeen, A., Zipori, D., and Kimchi, A. 1986. Autocrine-related interferon controls c-*myc* suppression and growth arrest during hematopoietic cell differentiation. *Cell* **46,** 31-40.
220. Ridley, A.J., Paterson, H.F., Nolde N., and Land, H. 1988. *ras* -mediated cell cycle arrest is altered by nuclear oncogenes to induce schwann cell transformation. *EMBO J.* **7,** 1635-1645.
221. Rosenbaum H., Webb, E., Adams, J.M., Cory, S., Harris, A.W. 1989. N-*myc* transgene promotes B lymphoid proliferation, elicits lymphomas and reveals cross-regulation with c-*myc*. *EMBO J.* **8,** 749-755.
222. Rouah, E., Wilson, D.R., Armstrong, D.L., and Darlington, G.J. 1989. N-*myc* amplification and neuronal differentiation in human primitive neuroectodermal tumors of the central nervous system. *Cancer Res.* **49,** 1797-1801.
223. Saksela, K., Mäkelä, T.P., Evan, G., and Alitalo, K. 1989. Rapid phosphorylation of the L-*myc* protein induced by phorbol ester tumor promoters and serum. *EMBO J.* **8,** 149-157.
224. Sap, J., Munoz, A., Damm, K., Ghysdael, J., Leutz, A., Beug, H., and Vennström, B. 1986. The c*erb* A protein is a high affinity receptor for thyroid hormone. *Nature* **324,** 635-640.
225. Sarid, J., Halazonetis, T.D., Murphy, W., and Leder, P. 1987. Evolutionary conserved regions of the human c-*myc* protein can be

uncoupled from transforming activity. *Proc. Natl. Acad. Sci. USA* **84,** 170-173.

226. Sassone-Corsi, P. and Borelli. 1987. Promoter trans-activation of protooncogenes c-*fos* and c-*myc*, but not c-Ha-*ras*, by products of adenovirus early region 1A. *Proc. Natl. Acad. Sci. USA* **84,** 6430-6433.
227. Sassone-Corsi, P., Lamph, W.W., Kamps, M., and Verma, I.M. 1988. *fos* -associated cellular p39 is related to nuclear transcription factor AP-1. *Cell* **54,** 553-560.
228. Schimke, R.T., Brown P.C., Kaufman, R.J., McGrogan, M., and Slate, D.L. 1980. Chromosomal and extrachromosomal localization of amplified dihydrofolate reductase genes in cultured mammalian cells. *Cold Spring Harbor Symp. Quant. Biol.* **45,** 785-797.
229. Schmid, P., Schulz, W.A., and Hameister, H. 1989. Dynamic expression pattern of the *myc* protooncogene in midgestation mouse embryos. *Science* **243,** 226-229.
230. Schwab, M., Varmus, H.E., Bishop, J.M., Grzeschik, K.-H., Naylor, S.L., Sakaguchi, A.Y., Brodeur, G., and Trent, J. 1984. Chromosomal localization in normal human cells and neuroblastomas of a gene related to c-*myc*. *Nature* **308,** 288-291.
231. Schwab, M., Varmus, H.E., and Bishop, J.M., 1985a. Human N-*myc* gene contributes to neoplastic transformation of mammalian cells in culture. *Nature* **316,** 160-162.
232. Schwab, M., Ramsay, G., Alitalo, K., Varmus, H.E., Bishop, J.M., Martinsson, T., Levan, G., and Levan, A. 1985b. Amplification and enhanced expression of the c-*myc* oncogene in mouse SEWA tumour cells. *Nature* **315,** 345-347.
233. Schwab, M. and Bishop, J.M. 1988. Sustained expression of the human protooncogene *MYCN* rescues rat embryo cells from senescence. *Proc. Natl. Acad. Sci. USA* **85,** 9585-9589.
234. Schwartz, R.C., Stanton, L.W., Riley, S.C., Marcu, K.B., and Witte, O.N. 1986. Synergism of v-*myc* and v-HA-*ras* in the *in vitro* neoplastic progression of murine lymphoid cells. *Mol. Cell. Biol.* **6,** 3221-3231.
235. Schweinfest, C.W., Fujiwara, S. Lau, L.F., and Papas, T.S. 1988. c-*myc* can induce expression of Go/G1 transition genes. *Mol. Cell. Biol.* **8,** 3080-3087.
236. Seeger, R., Brodeur, G., Sather, H., Dalton, A., Siegel, S., Wong, K., and Hammond, O. 1985. Association of multiple copies of the N-*myc* oncogene with rapid progression of neuroblastomas. *New Engl. J. Med.* **313,** 1111-1116.
237. Selten, G., Cuypers, H.T., Zijlstra, M., Melief, C., and Berns, A. 1984. Involvement of c-*myc* in MuLV-induced T-cell lymphomas in mice. Frequency and mechanisms of activation. *EMBO J.* **3,** 3215-3222.
238. Semsei, I., Ma, S., and Cutler, R.G. 1989. Tissue and age specific expression of the *myc* proto-oncogene family throughout the life span of the C57BL/6J mouse strain. *Oncogene* **4,** 465-470.
239. Setoyama, C., Frunzion R., Liau, G. Mudryj, M, and deCrombrugghe, B. 1986. Transcriptional activation encoded by the v-*fos* gene. *Proc. Natl. Acad. Sci. USA* **83,** 3213-3217.
240. Shaw, A.P.W., Poirier, V., Tyler, S., Mott, M., Berry, J., and Maitland, N.J. 1988. Expression of the N-*myc* oncogene in Wilms´ tumor and related tissues. *Oncogene* **3,** 143-149.
241. Shilo, B.-Z., and Weinberg, R.A. 1981. DNA sequences homologous to vetebrate oncogenes are conserved in *Drosophila melanogaster. Proc. Natl., Acad. Sci. USA* **78,** 6789-6792.
242. Shtivelman, E., and Bishop, J.M. 1989. The *PVT* gene frequently amplifies with *MYC* in tumor cells. *Mol. Cell. Biol.* **9,** 1148-1154.
243. Shtivelman, E., Henglein, B., Groitl, P., Lipp, M., and Bishop, J.M. 1989. Identification of a human transcription unit affected by the variant chromosomal translocations 2;8 and 8;22 of Burkitt lymphoma. *Proc. Natl. Acad. Sci. USA* **86,** 3257-3260.
244. Sinn, E., Muller, W., Pattengale, P., Tepler, I., Wallace, R., and Leder, P. 1987. Coexpression of MMTV/v-Ha-*ras* and MMTV/c-*myc* genes in transgenic mice: synergistic action of oncogenes *in vivo*. *Cell* **49,** 465-476.
245. Sklar, M.D., Tereba, A., Chen, B.D.-M., and Walker, W.S. 1985. Transformation of mouse bone marrow cells by transfection with a human oncogene related to c-*myc* is associated with the endogenous production of macrophage colony stimulating factor 1. *J. Cell. Physyol.* **125,** 403-412.
246. Slamon, D., T. Bone, R. Seeger, D. Keith. V. Chazin, H. Lee, and L. Souza. 1986. Identification and characterization of the protein encoded by the human N-*myc* oncogene. *Science* **232,** 768-772.

247. Small, M.B., Hay, N., Schwab, M., and Bishop, J.M. 1987. Neoplastic transformation by the human gene N-*myc*. *Mol. Cell. Biol.* **7,** 1638-1645.
248. Smeland, E., Godal, T., Rund, E., Beiske, K., Funderud, S., Clark, E.A., Pfeifer-Ohlsson, S., and Ohlsson, R. 1985. The specific induction of *myc* proto-oncogene expression in normal human B cells is not a sufficient event for aquisition of competence to proliferate. *Proc. Natl. Acad. Sci. USA* **82,** 6255-6259.
249. Sorrentino, V., Drozdoff, V., MuKinney, M.D., Zeitz, L., and Fleissner, E. 1986. Potentiation of growth factor activity by exogeneous c-*myc* expression. *Proc. Natl. Acad. Sci. USA* **83,** 8167-8171.
250. Spandidos, D.A., and Wilkie, I.M. 1984. Malignant transformation of early passage rodent cells by a single mutant oncogene. *Nature* **310,** 469-475.
251. Spector, D.L., Watt, R.A., and Sullivan, N.F. 1987. The v- and c-*myc* oncogene proteins colocalize *in situ* with small nuclar ribonucleoprotein particles. *Oncogene* **1,** 5-12.
252. Stanton, L.W., Schwab, M., and Bishop, J.M. 1986. Nucleotide sequence of the human N-*myc* gene. *Proc. Natl. Acad. Sci. USA* **83,** 1772-1776.
253. Stehelin, D., Varmus, H.E., Bishop, J.M., and Vogt, P.K. 1976. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature* **260,** 170-173.
254. Stern, D.F., Roberts, A.B., Roche, N.S., Sporn, M.B., and Weinberg, R.A. 1986. Differential responsiveness of *myc* - and *ras* - transfected cells to growth factors: selective stimulation of *myc* -transfected cells by epidermal growth factor. *Mol. Cell. Biol.* **6,** 870-877.
255. Stone, J., T. Lange, G. Ramsay, E. Jakobovits, J.M. Bishop, H. Varmus, and W. Lee. 1987. Definitions of regions in human c-*myc* that are involved in transformation and nuclear localization. *Mol. Cell. Biol.* **7,** 1697-1709.
256. Sugiyama, H., Silva, S., Babonits, M., Potter, M., Wiener, F., and Klein, G. 1989. A murine plasmacytoma with a variant (15;16) (D2/3;B1/2) translocation that involves the c-*myc* and lambda light chain gene-carrying chromosomes. *Cancer Genet. Cytogenet.* In press.
257. Suarez, H.G., Nardeux, P.C., Andeol, Y., and Sarasin, A. 1987. Multiple activated oncogenes in human tumors. *Oncogene Res.* **1,** 201-207.
258. Sullivan, N.F., Watt, R.A., Delannoy, M.R., Green C.L., and Spector, D.L. 1986. Colocalization of the oncogene protein and small nuclear ribonucleoprotein particles. *Cold Spring Harb. Symp. Quant. Biol.* **51,** 943-947.
259. Sümegi, J., Spira, J., Bazin, H., Szpirer, J., Levan, G., and Klein, G. 1983. Rat c-*myc* oncogene is located on chromosome 7 and rearranges in immunocytomas with t(6:7) chromosomal translocation. *Nature* **306,** 497-498.
260. Sümegi, J., T. Hedberg, M. Björkholm, T. Godal, H. Mellsted, M.G. Nilsson, C. Perlman, and G. Klein. 1985. Amplification of the c-*myc* oncogene in human plasma-cell leukemia. *Int. J. Cancer* **36,** 367-371.
261. Sümegi, J., Pear, W.S., Nelson, S., Wahlström, G., Ingvarsson, S., Melani, C., Szeles, A., Wiener, F., and Klein., G. 1987. Chromosome abberation and oncogene activation in two histologically related human and rat B-cell tumors. *Viral Carcinog.* **24,** 241-251.
262. Swartwout, S.G., and Kinniburgh, A.J. 1989. C-*myc* RNA degradation in growing and differentiating cells: Possible alternate pathways. *Mol. Cell. Biol.* **9,** 288-295.
263. Tabor, C.W. and Tabor, W. 1984. Polyamines. *Annu. Rev. Biochem.* 53, 749-790.
264. Taparowsky, E., Shimizu, K., Goldfarb, M., and Wiegler, M. 1983. Structure and activation of the human N-*ras* gene. *Cell* **34,** 581-586.
265. Taub, R., Moulding, C., Battey, J., Murphy, W., Vasicek, T., Lenoir, G.M., and Leder, P. 1984. Activation and somatic mutation of the translocated c-*myc* gene in Burkitt lymphoma cells. *Cell* **36,** 339-348.
266. Taya, Y., S. Mizusawa, and S. Nishimura. 1986. Nucleotide sequence of the coding region of the mouse N-*myc* gene. *EMBO J.* **5,** 1215-1219.
267. Thalmeier, K., Synovzik, H., Mertz, R., Winnacker, E.-L. and Lipp, M. 1989. Nuclear factor E2F mediates basic transcription and *trans* -activation by E1a of the human *MYC* promoter. *Genes Dev.* 3, 527-536.
268. Thiele, C.J., Reynolds, C.P., and Israel, M.A. 1985. Decreased expression of N-*myc* precedes retinoic acid-induced morphological differentiation of human neuroblastoma. *Nature* **313,** 404-406.

269. Thompson, C.B., Challoner, P.B., Neiman, P.E., and Groudine, M. 1985. Levels of c-*myc* oncogene mRNA are invariant throughout the cell cycle. *Nature* **314,** 363-366.
270. Thompson, T.C., Southgate, J., Kitchener, G., and Land, H. 1989. Multistage carcinogenesis induced by *ras* and *myc* oncogenes in a reconstituted organ. *Cell* **56,** 917-930.
271. Tian, S.-S. and Faust, C. 1987. Rearrangement of rat immunoglobulin E heavy-chain and c-*myc* genes in the B-cell immunocytoma IR162. *Mol. Cell. Biol.* **7,** 2614-2619.
272. Timmers, H.T.M., DeWit, D., Bos, J.L., and Eb, A.J. 1988. E1A products of adenovirus reduce the expression of cellular proliferation-associated genes. *Oncogene Res.* **3,** 67-76.
273. Torelli, G., Selleri, L., Donelli, A., Ferrari, S., Emilia, G., Venturelli, D., Moretti, L., and Torelli, U. 1985. Activation of c-*myb* expression by phytohemaglutinin stimulation in normal human T lymphocytes. *Mol. Cell. Biol.* **5,** 2874-2877.
274. Ueno, K., Katoh, K., and Kondoh, H. 1988. Subnuclear localization and antitransforming activity of N-*myc* :ß-Galactosidase fusion proteins. *Mol. Cell. Biol.* **8,** 4529-4532.
275. VanBeneden, R.J., Watson, D.K., Chen, T.T., Lautenberger, J.A., and Papas, T.S. 1986. Cellular *myc* (c-*myc*) in fish (rainbow trout): Its relationship to other vertebrate *myc* genes and to the transforming genes of the MC29 family of viruses. *Proc. Natl. Acad. Sci. USA* **83,** 3698-3702.
276. VanLohuizen, M., Breuer, M., and Berns, A. 1989a. N-*myc* is frequently activataed by proviral insertion in MuLV-induced T cell lymphomas. *EMBO J.* **8,** 133-136.
277. VanLohuizen, M., Verbeek, S., Kirmpenfort, P., Domen, J., Saris, C., Radaszkiewicz, T., and Berns, A. 1989b. Predisposition to lymphomagenesis in *pim* -1 transgenic mice: cooperation with c-*myc* and N-*myc* in murine leukemia virus-induced tumors. *Cell* **56,** 673-682.
278. Vennström, B., Sheiness, D., Zabielski, J., and Bishop, J.M. 1982. Isolation and characterization of c-*myc*, a cellular homolog of the oncogene (v-*myc*) of avian myelocytomatosis virus strain 29. *J. Virol.* **42,** 773-779.
279. Villares, R., and Cabrera, C.V. 1987. The *achaete-scute* gene complex of *D. melanogaster*: Conserved domains in a subset of genes required for neurogenesis and their homology to *myc. Cell* **50,** 415-424.
280. Villeneuve, L, Rassart, E., Jolicoeur, P., Graham M., and Adams, J.M. 1986. Proviral integration site *Mis* -1 in rat thymomas corresponds to the *pvt* - 1 translocation breakpoint in murine plasmacytomas. *Mol. Cell. Biol.* **6,** 1834-1837.
281. Wabl, M.R. and Burrows, P.D. 1984. Expression of immunoglobulin heavy chain at a high level in the absence of a proposed immunglobulin enhancer element in *cis. Proc. Natl. Acad. Sci. USA* **81,** 2452-2455.
282. Wahlström, G., Pear, W.S., Steen, M.-L., Szpirer, J., Levan, G., Klein, G., and Sümegi, J. 1988. Localization of the rat immunoglobulin lambda light chain locus to chromosome 11. *Immunogenetics* **28,** 182-183.
283. Webb, E., Adams, J.M., and Cory, S. 1984. Variant (6;15) translocation in a murine plasmacytoma occurs near an immunoglobulin k gene but far from the *myc* oncogene. *Nature* **312,** 777-779.
284. Weinberg, R.A., 1985. The action of oncogenes in the cytoplasm and nucleus. *Science* **230,** 770-776.
285. Weinberger, C., Thompson, C., Ong, E., Gruol, D., and Evans, R. 1986. The c-*erb* A gene encodes a thyroid hormone receptor. *Nature* **324,** 641-646.
286. Weisinger, G., Remmers, E.F., Hearing, P., and Marcu, K.B. 1988. Multiple negative elements upstream of the murine c-*myc* gene share nuclear factor binding sites with SV40 and polyoma enhancers. *Oncogene* **3,** 635-646.
287. Westin, E.H., Wong-Staal, F., Gelman, E.P., Dalla-Favera, R., Papas, T., S., Lautenberger, J.A., Eva, A., Reddy, E.P., Tronick, S.R., Aaronson, S.A., and Gallo, R.C. 1982. Expression of cellular homologoues of retroviral *onc* genes in human hematopoietic cells. *Proc. Natl. Acad. Sci. USA* **79,** 2490-2494.
288. Wickström, E.L., Bacon, T.A., Gonzalez, A., Freeman, D.L., Lyman, G.H., and Wickström, E. 1988. Human promyelocytic leukemia HL-60 cell proliferation and c-*myc* protein expression are inhibited by and antisense pentadecadeoxynucleotide targeted agains c-*myc* mRNA. *Proc. Natl. Acad. Sci. USA* **85,** 1028-1032.
289. Wiener, F., Babonits, M., Spira, J., Klein, G., and Bazin, H. 1982. Non-random chromosomal changes involving chromosomes 6 and 7 in spontaneous rat

immunocytomas. *Int. J. Cancer* **29,** 431-437.

290. Wiman, K.G., Clarkson, B., Hayday, A.C., Saito, H., Tonegawa, S., and Hayward, W.S. 1984. Activation of a translocated c-*myc* gene: Role of structural alterations in the upstream region. *Proc. Natl. Acad. Sci. USA* **81,** 6798-6802.
291. Winqvist, R., Saksela, K., and Alitalo, K. 1984. The *myc* proteins are not associated with chromatin in mitotic cells. *EMBO J.* **3,** 2947-2950.
292. Wirschubsky, Z., Ingvarsson, S., Carstenssen, A., Wiener, F., Klein, G., and Sümegi, J. 1985. Gene localization on sorted chromosomes: Definitive evidence on the relative positioning of genes participating in the MPC-associated typical translocation. *Proc. Natl. Acad. Sci. USA*, **82,** 6975-6979.
293. Wright, S. and Bishop, J.M. 1989. DNA sequences that mediate attenuation of transcription from the mouse protooncogene *myc*. *Proc. Natl. Acad. Sci. USA* **86,** 505-509.
294. Yancopoulos, G.D., Nisen, P.D., Tesfaye, A., Kohl, N.E., Goldfarb, M.P., and Alt, F.W. 1985. N-*myc* can cooperate with *ras* to transform normal cells in culture. *Proc. Natl. Acad. Sci. USA* **82,** 5455-5459.
295. Yang, J.-Q., Bauer, S.R., Mushinski, J.F., and Marcu, K.B. 1985. Chromosome translocation clustered 5′ of the murine c-*myc* gene qualitatively affect promoter usage: implications for the site of normal c-*myc* regulation. *EMBO J.* **4,** 1441-1447.
296. Yanofsky, C. 1981. Attenuation in the control of expression of bacterial operons. *Nature* **289,** 751-758.
297. Zimmerman, K.A., Yancopoulos, G.D., Collum, R.D., Smith, R.K., Kohl, N.E., Denis, K.A., Nau, M.M., Witte, O.N., Toran-Allerand, D., Gee, C.E., Minna, J.D., and Alt, F.W. 1986. Differential expression of *myc* family genes during murine development. *Nature* **319,** 780-783.