



# The *myb* gene family in cell growth, differentiation and apoptosis

Il-Hoan Oh<sup>1,2</sup> and E Premkumar Reddy<sup>\*1</sup>

<sup>1</sup>Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, 3307 North Broad Street, Philadelphia, Pennsylvania, PA 19140, USA

The *myb* gene family consists of three members, named A, B and *c-myb* which encode nuclear proteins that function as transcriptional transactivators. Proteins encoded by these three genes exhibit a tripartite structure with an N-terminal DNA-binding domain, a central transactivation domain and a C-terminal regulatory domain. These proteins exhibit highest homology in their DNA binding domains and appear to bind DNA with overlapping sequence specificities. Transactivation by *myb* gene family varies considerably depending on cell type and promoter context suggesting a dependence on interaction with other cell type specific co-factors. While the C-terminal domains of A-Myb and c-Myb proteins exert a negative regulatory effect on their transcriptional transactivation function, the C-terminal domain of B-Myb appears to function as a positive regulator of this activity. One or more of these proteins interact with other transcription factors such as Ets-2, CEBP and NF-M. In addition, expression of these genes is cell cycle-regulated and inhibition of their expression with anti-sense oligonucleotides has been found to affect cell cycle-progression, cell division and/or differentiation. Members of the *myb* gene family exhibit different temporal and spatial expression patterns suggesting a distinctive function for each of these genes. Gene knockout experiments show that these genes play an essential role in development. Loss of *c-myb* function results in embryonic lethality due to failure of fetal hepatic hematopoiesis. A-*myb* null mutant mice, on the other hand are viable but exhibit growth abnormalities, and defects in spermatogenesis and female breast development. While the role of *c-myb* in oncogenesis is well established, future experiments are likely to provide further clues regarding the role of A-*myb* and B-*myb* in tumorigenesis.

**Keywords:** A-*myb*; B-*myb*; *c-myb*; *v-myb*; cell growth; differentiation

## Discovery of *myb*

The *myb* oncogene is the transforming gene of Avian myeloblastosis virus (AMV), which was originally isolated by Hall *et al.* in 1941. Several years later, another virus, also carrying the *myb* oncogene was isolated in Bulgaria and was found to induce in quails, a leukemia involving erythroid and myeloid cells (Ivanov *et al.*, 1964; Nedyalkov *et al.*, 1975). In

chickens, the same virus can induce only an erythroblastic leukemia. When white blood cells from the leukemic chickens were cultured *in vitro* and examined for surface markers, about half of them expressed erythroid antigens and the other half showed myeloid markers suggesting that E26 virus induces both an erythroblastic and myeloid leukemia.

Subsequent molecular cloning and sequence analysis of the two viral genomes revealed that AMV was generated by recombination between the replication-competent myelo-blastosis-associated virus type 1 (MAV-1) and proto-oncogene sequences (*c-myb*) found in normal chicken cellular DNA (reviewed by Baluda and Reddy, 1994). The *v-myb*<sup>AMV</sup> oncogene product, a 45 kD protein, was found to be a truncated version of the 75 kD c-Myb protein (Baluda and Reddy, 1994 and references therein). The protein contained 6 and 11 amino acids from the viral gag and env, respectively, in addition to 370 amino acids encoded by *c-myb*. In the case of *v-myb*<sup>E26</sup> the viral genome was found to code for a 135 kD protein which is a gag-Myb-Ets-1 fusion protein. Thus, this virus appears to have transduced two different proto-oncogenes leading to the formation of an acute transforming virus (Nunn *et al.*, 1983; Leprince *et al.*, 1983).

Following the discovery and molecular cloning of the *v-myb* oncogene, it has been possible to examine the role of the normal cellular counterpart of this oncogene in cell growth, differentiation and development. These studies have revealed that the *c-myb* gene is highly conserved through evolution and is present in all vertebrate and some invertebrate species examined (Lipsick, 1996). Proteins encoded by the viral as well as the cellular *myb* genes are localized in the nucleus, exhibit a sequence-specific DNA-binding activity and appear to function as regulators of transcription. In this review, we will only discuss the structure and function of mammalian and avian *myb* genes as their role in cell growth and neoplasia have been best studied. While the role of *c-myb* gene in cell growth and neoplastic transformation has been best studied, recent studies have revealed that the *myb* gene family consists of two additional members which have been designated as A-*myb* and B-*myb*. A-*myb* and B-*myb* were identified by screening a human T-cell cDNA library by low stringency hybridization with a *c-myb* probe (Nomura *et al.*, 1988). The homologues of these A-*myb* and B-*myb* genes have been identified in several vertebrates (Bouwmeester *et al.*, 1994; Lam *et al.*, 1992; Mettus *et al.*, 1994; Trauth *et al.*, 1994; Foos *et al.*, 1992; Sleeman, 1993). These *myb* family genes share extensive homology in their sequence but seem to be expressed in different tissues, though a number of tissues seem to express more than one member of the

\*Correspondence: E Premkumar Reddy

<sup>2</sup>Current address: Terry Fox Laboratory, BC Cancer Agency, 601 W. 10th Avenue, Vancouver, British Columbia, V5Z 1L3, Canada

*myb* gene family. A number of recent studies have begun to address the question as to whether they perform similar functions in cells where they are expressed or whether they have distinctive biological and biochemical functions.

### Products of *myb* gene family

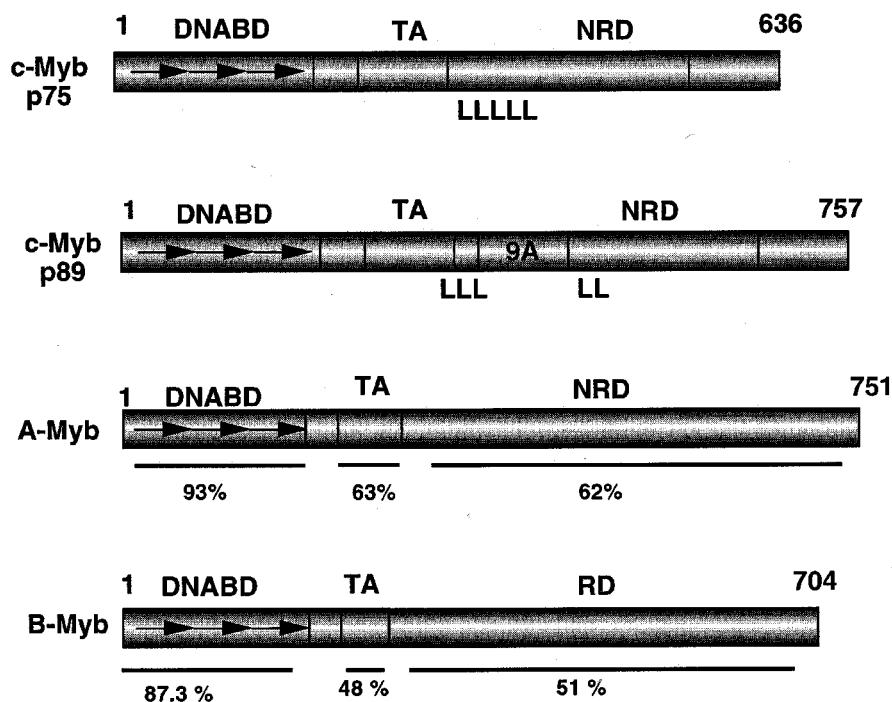
The major translational product of the *c-myb* proto-oncogene is a 75 kD nuclear protein with 636 amino acids which is expressed in most hematopoietic tissues (Gonda *et al.*, 1982; Westin *et al.*, 1982). In addition to this 75 kD protein product, another translational product of 89 kD was found to be encoded by *c-myb* in several avian, murine and human hematopoietic cells (Dudek and Reddy, 1989a,b; Dasgupta *et al.*, 1989). This 89 kD protein is translated from an alternatively spliced mRNA encoded by the *c-myb* gene which results in the addition of 363 base pairs between the exons 9 and 10. This region has been designated as exon 9A (Rosson *et al.*, 1987; Shen-Ong, 1987). These translated proteins were found to be localized in the nucleus (Dudek and Reddy, 1989a,b) and function as transcriptional activators with sequence specific DNA binding activities (Sakura *et al.*, 1989; Weston and Bishop, 1989).

Like *c-myb*, *A-myb* and *B-myb* also encode for nuclear proteins of approximately 95 kD and 93 kD, which contain 751 and 704 amino acids respectively (Figure 1). Interestingly, the exon 9A sequence of *c-myb* is also found in *A-myb* and *B-myb*. PCR analysis revealed the existence of a transcript of *B-myb* lacking the Exon 9A region in a broad spectrum of cells (Kamano *et al.*, 1995). However, a protein product of B-Myb that lacks this region has not so far been

identified and awaits further experimental evidence. In *A-myb*, the alternatively spliced product lacking the exon 9A region has not been found by PCR-based analyses. Instead, differentially spliced products with a truncation in the region corresponding to 2199–2378 bases has been identified by PCR (Mettus *et al.*, 1994). Again, like with *B-myb*, there has been no experimental evidence to suggest that these transcripts are indeed translated into protein products in cells expressing these mRNAs. While the three genes exhibit considerable homology, they show very different patterns of expression. Thus, while *c-myb* is primarily expressed in the immature hematopoietic cells (Gonda *et al.*, 1982; Westin *et al.*, 1982; Duphrey and Boettiger, 1985), *A-myb* is predominantly expressed in the male germ cells and breast epithelial cells of pregnant mice (Mettus *et al.*, 1994; Toscani *et al.*, 1997). Also, low levels of *A-myb* expression could be detected in ovaries, developing brain as well as B-cells of spleen germinal centers (Trauth *et al.*, 1994; Mettus *et al.*, 1994; Foos *et al.*, 1994). In contrast to the tissue-specific expression of *A-myb* and *c-myb*, the *B-myb* gene expression seems to be ubiquitous (Nomura *et al.*, 1988).

### Unique DNA binding motif of Myb proteins

The three Myb proteins share extensive amino acid sequence homology and several structural and functional features can be identified in these proteins. The first one-third of the molecule consists of an unusual structure of three tandem 50 amino acid direct repeats termed R1, R2, and R3 (Saikumar *et al.*, 1990; Sakura *et al.*, 1989; Weston and Bishop, 1989). This structure is known to mediate binding of the Myb protein to



**Figure 1** Structural comparison of Myb gene family products. Schematic structure of A-, B- and c-Myb proteins is represented. The numbers above each diagram is amino acid residues in each corresponding region. Thick bar represents the region of homology and the numbers below are % homology to c-Myb in each domain. DNBD, DNA binding domain; NRD, negative regulatory domain; RD, regulatory domain; TA, transactivation domain, leucine zipper domain is shown as LLLLL

DNA, and is highly conserved evolutionarily (Lipsick, 1986; Mettus *et al.*, 1994). Two important features have been noted within these tandem repeats. First, there is a periodic occurrence of tryptophans (Anton and Frampton, 1988); each of the three repeats has three tryptophans which are separated by 18 or 19 amino acid residues. All of these are conserved between mouse, human, chicken, and drosophila *c-myb*, corn c1 and yeast Bas1 as well as A-*myb* and B-*myb* (Nomura *et al.*, 1988; Mettus *et al.*, 1994; Lipsick, 1996). Deletion analysis showed that, among these three repeats, R2 and R3 are absolutely required for complex formation of Myb with DNA while R1 is completely dispensable (Howe *et al.*, 1990; Saikumar *et al.*, 1990). Subsequent study suggested that R1 is involved in stabilization of the complex between DNA and R2R3 sequences (Tanikawa *et al.*, 1993). The R2 R3 fragment was predicted to contain two consecutive helix–turn–helix (HTH) motifs with unconventional turns. Mutagenesis of the amino acid residues at positions corresponding to the DNA-contacting residues in other HTH containing proteins abolished specific DNA binding supporting the prediction (Gabrielsen *et al.*, 1991). Interestingly, mutagenesis of the tryptophan residues into glycine, proline or arginine abolished the DNA binding activity of c-Myb, whereas replacement with other aromatic residue or leucine or alanine did not appreciably affect the DNA binding (Saikumar *et al.*, 1990). Based on these results, it was suggested that tryptophan residues form a hydrophobic scaffold which maintains the helix–turn–helix motif (Saikumar *et al.*, 1990). In this report, it was also observed that arginine and lysine residues that flank the last tryptophan residue were critical for DNA binding as mutation of these residues abolished the DNA binding activity of c-Myb protein (Saikumar *et al.*, 1990).

The NMR study of the three repeat domain complexed with the DNA revealed that R2 and R3 is packed in the major groove to bind the double helical DNA cooperatively and that the Asn-183 (R3), Lysine-182 (R3) and Lys-128 (R2) specifically interact with the first three bases of AACNG supporting the previous observation (Ogata *et al.*, 1994). Recently another NMR study suggested that a cavity inside of hydrophobic core of R2 is important for DNA binding and transactivation (Ogata *et al.*, 1996). Therefore the tryptophan repeat with regular spacing is functionally significant and structurally unique. This structure is essentially conserved in A-Myb and B-Myb with almost identical sequence in R2 and R3 (reviewed by Introna *et al.*, 1994; Kanei-Ishii *et al.*, 1996). In addition, several Myb-related proteins and c-DNAs have been found from human, *Drosophila*, yeast, and *Zea mays* with the DNA-binding domain being the most conserved region (reviewed by Luscher and Eisenman, 1990). Thus the DNA binding motif with tryptophan repeat is a characteristic feature which defines the whole Myb family proteins. Similar tryptophan repeats have also been found in the Ets family of genes suggesting functional similarity between the *myb* family and Ets family (Wasylyk *et al.*, 1993).

Although A-, B- and c-*myb* genes are believed to code for transcriptional regulators, it should be noted that the 'Myb domain' has been identified in proteins which do not appear to function as transcriptional

regulators. For example, each end of a given chromosome is stabilized by structures termed telomeres, composed of long arrays of TTAGGG repeats that form nucleoprotein complexes. Recently two important components of this nucleoprotein complex have been identified, namely TTAGGG repeat binding factors 1 and 2 (TRF-1 TRF-2). Interestingly, the C-terminal domain of both these factors exhibit striking homology to Myb DNA binding domain (*myb*-like domain) which appears to mediate the sequence-specific DNA binding of these proteins (Broccoli *et al.*, 1997; Cooper *et al.*, 1997; Konig *et al.*, 1998). Another interesting *myb*-like domain has been recently identified in a novel transcription factor, the Cyclin D-interacting *myb*-like protein (designated DMP1). This protein's main function appears to be to prevent the entry of cell cycle into S phase and its transactivation potential has been found to be specifically inhibited by D-type cyclins, but not by A, E, or B-type cyclins (Hirai and Sherr, 1996; Inoue and Sherr, 1998; Inoue *et al.*, 1998). Thus, the repertoire of *myb*-related genes as defined by the DNA binding motif of Myb ('myb domain') is extensive. This review will focus exclusively on A-, B- and c-*myb* genes as they have been the best studied members of the family and play a critical role in cell growth and neoplasia.

#### Sequence recognition by Myb DNA binding motif

The study of Myb/DNA interactions using the DNA binding domain of v-Myb showed that this protein binds specifically to PyAACG/TG *in vitro* (Bidenkapp *et al.*, 1988). This consensus sequence is also present in the target promoters of Myb such as *mim-1* identified in E26 transformed promyelocytic cells and MBS1 of the SV40 enhancer sequences (Ness *et al.*, 1989; Nakagoshi *et al.*, 1990). The identified Myb binding sequences (MBS and MRE) showed a similar binding affinity for A-Myb and B-Myb (Golay *et al.*, 1994; Howe and Watson, 1991; Mizuguchi *et al.*, 1990). However, some distinct preferences for nucleotides flanking the core binding site for individual Myb proteins have been reported implicating potential subtle distinction for B-Myb and c-Myb binding sequences (Howe and Watson, 1991; Mizuguchi *et al.*, 1990). In addition, the *in vitro* mobility shift assays and methylation-interference assays combined with effector reporter analysis showed that B-Myb or v-Myb<sup>AMV</sup> have less flexibility in sequence recognition of second half of consensus YAACNG suggesting that c-Myb may recognize a more extensive repertoire of promoters. Interestingly, *in vitro* translated full-length B-Myb did not bind to Myb binding site in the SV40 promoter, but the C-terminal truncation mutants of B-Myb or B-Myb DNA binding domain fused to c-Myb were able to bind the same sequences suggesting that C-terminus of B-Myb exerts additional limitation to DNA binding (Watson *et al.*, 1993). Third, the NMR structure for the DNA binding domain of B-Myb revealed that despite extensive sequence similarity in the R2 and R3 regions, the C-terminal region of B-Myb R2 shows a poorly defined structure, reflecting the existence of multiple conformations in slow to intermediate exchange (McIntosh *et al.*, 1998). This contrasts with the tertiary structure reported for c-Myb

R2R3, in which both R2 and R3 have the same folding and the C-terminal region of R2 forms a stable, well-defined helix (Ogata *et al.*, 1994). It has been proposed that conformational instability of part of the DNA-binding motif is a way of increasing the specificity of Myb proteins for a relatively short (6 bp) DNA target site by reducing their affinity for non-specific DNA sequences compared to specific sites (Carr *et al.*, 1996). Thus each sub-family of Myb proteins may recognize overlapping ranges of target promoters containing core binding sequences, but still with distinctive preference depending on sequence context of the target sequences. This observation provides an attractive model for distinctive biological function of *myb* family genes. Thus all three Myb proteins may recognize regulatory regions of an overlapping range of target promoters with a certain degree of distinctive preferences among the various members of the family.

### Transactivation by Myb family of proteins

The deletional and linker insertional analyses of c-Myb and v-Myb have revealed a transactivation domain downstream of the DNA binding domain encompassing a stretch of 52 amino acids in human-Myb (aa 275–327) and 85 amino acids (aa 241–325) in mouse c-Myb (Sakura *et al.*, 1989; Weston and Bishop, 1989; Ibanez and Lipsick, 1990; Lane *et al.*, 1990; Kalkbrenner *et al.*, 1990). The transactivation domain of c-Myb contains clusters of acidic residues similar to those found in other transactivator domains (reviewed by Ptashne, 1988). However, the deletion of 11 amino acids within the transactivation domain, which has little effect on the overall charge of this region, abolishes the Myb transactivating activity (Weston and Bishop, 1989) suggesting that the acidity of the amino acid residues is not an essential part of the domain.

Although the transactivation domains of c-Myb and A-Myb show sequence differences, the A-Myb transactivation domain was also shown to have clusters of acidic amino acids, analogous to the c-Myb transactivation domain. In accordance with the structural similarity, the transactivation potential of A-Myb could be demonstrated with various forms of Myb-responsive promoters (Foos *et al.*, 1994; Golay *et al.*, 1994; Takahashi *et al.*, 1995; Trauth *et al.*, 1994; Oh and Reddy, 1997). However, the transactivation potential of A-Myb has been shown to vary depending on species and experimental system. Thus, the human A-Myb was reported to have 6–10 times higher transactivation potential than that of c-Myb (Golay *et al.*, 1994) whereas the transactivation potential for chicken A-*myb* was comparable to that of c-*myb* (Foos *et al.*, 1994). In contrast, transactivation potential of murine A-*myb* was much weaker (3–5-fold) compared to c-*myb* (10–15-fold) in murine NIH3T3 cells (Oh and Reddy, 1997) as well as chicken QT6 cells (Ziebold and Klempnauer, 1997). While such differences in transactivation properties of A-*myb* could be attributed to many variables such as species, cell types or promoters, another line of evidence suggests that the transactivation potential may be dependent on the nature of Myb-interacting proteins present in cells used in these assays. Thus, when the transactivation potential of A-Myb was studied in B- and T-cell lines, A-*myb* was found to actively

transactivate transcription only in B-cell lines but not in T-cell lines. Interestingly, a nuclear protein of 110 kD present in B-cell lines was found to interact with the DNA binding domain of A-*myb* which was absent in T-cell lines where A-*myb* was inactive as a transcriptional transactivator. These results suggest that interaction with such tissue-specific factors could contribute to the observed differences in A-*myb* transactivating potential (Ying *et al.*, 1997).

In contrast to c-Myb and A-Myb, transactivation properties of B-Myb have been more controversial. The transactivation domain of B-*myb* contains clusters of acidic residues but bears little homology to that of c-Myb. In addition, the B-Myb transactivation domain failed to transactivate promoters containing GAL4 binding sites when fused to the DNA binding domain of GAL4, whereas the transactivation domain of c-Myb functions in conjunction with GAL4 DNA binding domain (Watson *et al.*, 1993). These results suggest that the transactivation domain identified in B-Myb is not functionally equivalent to that of c-Myb. Similarly, chicken and mouse B-Myb proteins did not show transactivation of promoters containing multiple Myb-binding sites (MBS) and co-transfection of B-*myb* with c-*myb* inhibited transactivation by c-*myb* of its target genes (Watson *et al.*, 1993; Foos *et al.*, 1992). Thus an antagonistic relationship between c-*myb* and B-*myb* has been noted suggesting that competition for common target genes may occur for a certain set of target genes. However, in other assay systems, B-*myb* transactivation was comparable to c-*myb* or even higher than that of c-Myb (Golay *et al.*, 1997; Oh and Reddy, 1997). Recently, a conserved region in the C-terminal domain of B-Myb was shown to interact with cell type-specific co-activators (Tashiro *et al.*, 1995) implicating that the observed differences in transactivation potential of B-Myb may in fact reflect the cell type-specific expression of these factors. This notion is further supported by the observation that increasing dose of B-*myb* expression plasmid increased B-*myb*-mediated transactivation of DNA topoisomerase II- $\alpha$  promoter up to 15-fold in HL-60 cells whereas the same dose of plasmid could achieve only a fivefold activation in HeLa cells (Brandt *et al.*, 1997), suggesting that availability of cellular co-activators may constitute a mechanism of regulating B-*myb* activity.

In addition to DNA-binding-dependent transactivation of reporter genes, DNA-binding-independent transactivation of HSP 70 promoters have been reported with c-Myb, A-Myb and B-Myb (Foos *et al.*, 1993, 1994). B-Myb, despite its inability to transactivate the MBS containing promoters, can transactivate the HSP70 promoter in a DNA binding-independent manner which suggests the existence of another mechanism of transactivation, though the precise mechanism is unclear. One possible explanation would be that another transcription factor binds to HSP70 promoter and interacts with Myb. A second possibility is that some repressors for HSP70 promoter are sequestered by Myb.

### C-terminal regulatory domain of Myb

The observation that the v-*myb* gene lacks the C- and N-terminal sequences encoded by c-*myb*, had suggested

early on that the deleted regions of c-Myb may function as negative regulators of its function. This was formally demonstrated first by Sakura *et al.* (1989) and Weston *et al.* (1989) who showed that deletion of C-terminal sequences of c-Myb leads to a substantial increase in the transactivational activity of the protein. Subsequent studies also demonstrated increased transformation activity of C-terminal truncation mutants of c-Myb (Grasser *et al.*, 1991; Gonda *et al.*, 1989; Hu *et al.*, 1991). Additionally, increase in the DNA binding activity of the C-terminus truncated Myb protein has also been reported (Ramsay *et al.*, 1991).

One of the interesting characteristics noted in the negative regulatory domain of c-Myb was a putative leucine zipper motif with one Isoleucine and three leucine residues (Bedenkapp *et al.*, 1988) resembling that of other leucine zipper domains seen in other DNA binding transcription factors (Landschulz *et al.*, 1988). While the precise role of this leucine zipper domain in c-Myb function is unclear, a role for this domain in the negative regulation of c-Myb has been proposed. This view is supported by the observation that a mutation of one or more leucines to proline residues has been shown to result in increased transactivation and transformation activities of the mutant molecules, a phenomenon similar to that seen with C-terminal truncation (Kanei-Ishi *et al.*, 1992). The precise mechanism by which the leucine zipper domain exerts its negative regulatory effect remains unresolved. It has been suggested that this region might mediate the formation of Myb homodimers which are unable to bind DNA thereby contributing to a negative regulatory effect (Nomura *et al.*, 1993). A second model proposes a role for the leucine zipper region in mediating interaction with other cellular proteins, leading to the negative regulation of c-Myb (Favier and Gonda, 1994). Recently, a gene that encodes a protein that can bind to the leucine zipper domain of c-Myb has been identified (Tavner *et al.*, 1998). However, this protein appears to associate with other leucine zipper proteins such as c-Jun and its role in the negative regulation of c-Myb needs to be further explored. In contrast to the above set of studies, results from another study show that deletion of the leucine zipper region has no effect on the transcriptional transactivation characteristics of the mutant protein. Instead, deletion of two other regions within the C-terminal domain abolished the negative regulatory effect of the C-terminal domain, leading to the suggestion that these domains might be more important for the negative regulation of c-Myb protein (Dubendorff *et al.*, 1992). It is interesting to note that the p89 form of the c-Myb protein lacks the putative leucine zipper domain, because of the insertion of exon 9A encoded sequences within the zipper domain, resulting in its disruption. p89Myb has recently been shown to exhibit higher transactivational activities compared to its p75 counterpart, further lending support to the hypothesis that the leucine zipper may play an important role in the negative regulation of c-Myb (Woo *et al.*, 1998).

The C-terminal region of c-Myb is also conserved in A-Myb and B-Myb with highest homology in the middle of C-terminus, the region which is also conserved in *Drosophila* and *Xenopus* Myb (Sleeman

1993; Katzen *et al.*, 1985). Deletion of this region from A-Myb or B-Myb genes had a strikingly different effect on the biochemical activities of the encoded proteins. Deletion of the C-terminal domain of A-Myb resulted in marked increase in its transactivation potential, suggesting that the C-terminus of A-Myb functions as a strong negative regulatory domain, similar to c-Myb (Oh and Reddy, 1997; Takahashi *et al.*, 1995). Furthermore, our results show that the C-terminal domains of c-Myb and A-Myb could be exchanged without affecting their negative regulatory function (Oh and Reddy, 1997). In sharp contrast to c-Myb and A-Myb, C-terminal deletion of B-Myb did not increase its transactivation potential but in fact drastically reduced the ability of this protein to transactivate transcription (Nakagoshi *et al.*, 1993; Oh and Reddy, 1998). In addition, replacement of the C-terminal domain of c-Myb with that of B-Myb resulted in an up-regulation of the transactivation potential of the chimeric protein. These results suggest that the C-terminus of B-Myb acts as a positive regulator of transcriptional transactivation function of B-Myb (Oh and Reddy, 1998). In accordance with this observation, the C-terminal domain of B-Myb was shown to interact with a cell type-specific co-factor suggesting that this domain might function as a mediator of protein-protein interactions (Tashiro *et al.*, 1995). Although the C-terminus of B-Myb appears to function as a positive regulatory domain, it was also shown to contain a sub domain which functions as a negative regulator. Thus, deletion of a small region of the C-terminal end (downstream of conserved region) was found to increase the transactivation potential of B-Myb (Lane *et al.*, 1997; Ziebold *et al.*, 1997). In both cases, deletion of further upstream C-terminus abolished the transactivating function of B-Myb, suggesting that C-terminus of B-Myb encodes multiple functional domains which exert different constraints on the biochemical activity of B-Myb protein.

### Regulation of Myb transactivation by post-translational modification

c-Myb protein is phosphorylated at multiple sites *in vivo*, and some of these sites seem to be of functional importance. Ser-11 and Ser-12 have been mapped as *in vivo* phosphorylation sites for chicken c-Myb. These sites are phosphorylated by casein kinase II *in vitro*. The c-Myb protein synthesized in bacteria, upon being phosphorylated by casein kinase II has been found to exhibit weaker DNA binding activity (Luscher and Eisenmann, 1990). The c-Myb mutant in which Ser-11 and -12 were replaced with Ala (Myb Ala-11/12), showed decreased DNA binding when compared to wild-type c-Myb. In addition, the difference observed between the wild-type and mutants was abolished by the presence of NF-M, which cooperates with Myb in transcription of Myb-responsive genes (Burk *et al.*, 1993; Mink *et al.*, 1996). Thus, CK-II phosphorylation of N-terminal Serines could exert a direct inhibitory effect on the biochemical activity of Myb by affecting its DNA binding activity or an indirect effect where such phosphorylation affects the interaction of this protein with other cellular factors (Oelgeschlager *et al.*, 1996).

Since casein kinase II is a pleiotrophic kinase with more than 300 substrates and phosphorylates Ser/Thr residues followed by multiple acidic residues (Pinna, 1994 and reference therein), it is possible that A-Myb, which has a serine residue at position 7 (Ser-7) followed by similar multiple acidic residues (EDED) is also phosphorylated by casein kinase II. However, B-Myb shows less conservation of the motif for casein kinase II, raising the possibility that its regulation could be different from that of A-*myb* and c-*myb*. In addition to casein kinase, multiple phosphorylation sites for p42MAPK have been identified in the C-terminus of c-Myb including Ser 528 residue. Replacement of Ser 528 with Ala resulted in 2–7-fold increase in transactivation activity of c-Myb (Aziz *et al.*, 1993, 1995). It is at present unclear whether A-*myb* and B-*myb* activities are regulated by Casein kinase and MAPK in a similar manner to that of c-*myb*.

### Regulation of Myb-mediated transcriptional activation by other transcription factors

Recent studies have demonstrated that c-Myb protein cooperates with a wide variety of other transcription factors in the transcriptional transactivation of target promoters. These factors include p300/CBP (Dai *et al.*, 1996), CCAAT binding protein (c/EBP) family such as NF-M/C-EBP-beta (Burk *et al.*, 1993; Mink *et al.*, 1996), and c/EBP-alpha (Klempt *et al.*, 1998), Ets family proteins such as Ets-2 (Dudek *et al.*, 1992), PU.1 along with c/EBP-alpha (Oelgeschlager *et al.*, 1996), or tetrameric complex GABP (Nuchprayoon *et al.*, 1997), runt homology domain AML-1 (Britos-Bray and Friedman, 1997; Zaiman and Lenz, 1996) which is the alpha subunit of core binding factor (CBF). Many of the Myb-responsive promoters were found to show enhanced transactivation, when these cooperating factors are co-expressed with c-Myb, while some of the promoters appear to be activated only in the presence of both transcription factors, as exemplified by activation of neutrophil elastase by c-Myb and AML protein (Britos-Bray and Friedman, 1997). In some cases, transcriptional cooperation was found to be required to overcome active repression of the promoter. Thus, ZEB, the vertebrate homologue of drosophila Zfh-1 (zinc finger/homeodomain), is an active transcriptional repressor which plays an important role in muscle differentiation (Postigo and Dean, 1997). Zfh is involved in the active repression of promoters like alpha-4 integrin in hematopoietic cells, and transcriptional repression by this factor can be overcome only by co-expression of c-Myb and Ets, but not by any one of these proteins by themselves (Postigo and Dean, 1997).

While c-Myb has been shown to cooperate with a number of transcription factors, only a limited number of studies have been performed to address the question of transcriptional cooperation of A-*myb* or B-*myb* with other transcriptional co-factors. Recently, it was shown that NF-M/c-EBP beta is able to cooperate with A-*myb* and B-*myb* as well as with c-Myb (Foos *et al.*, 1994; Mink *et al.*, 1996). Similarly, P300/CBP was shown to physically associate with the transactivation domain and the C-terminal flanking region of c-Myb

and A-Myb resulting in transcriptional synergy (Dai *et al.*, 1996; Facchinetti *et al.*, 1997; Kiewitz and Wolfes, 1997).

### Ets-2

The Ets oncogene was first identified as one of the transforming genes of E26 virus, which codes for a fusion gene, gag-*myb-ets* (Leprince *et al.*, 1983; Nunn *et al.*, 1983). The *ets* family consists of at least 13 genes including *ets-1*, *ets-2*, Elk-1, SAP-1 and Elf-1. Among these, *ets-1* constitutes the gene that has been transduced by E26 (reviewed by Macleod *et al.*, 1992). The *ets* family of genes code for proteins that are characterized by the presence of a conserved DNA binding domain, called the Ets domain, which itself is characterized by the presence of three tryptophan residues which are spaced 18–19 amino acids apart, a feature also seen in c-Myb. The *ets* family of genes function as transcriptional activators and bind to *ets* binding sequence (EBS) GGA, which is the core motif. In many cases, the Ets proteins are involved in protein-protein interactions with other non-Ets transcription factors and the cooperative interaction is often regulated by extracellular signals (Janknecht and Nordheim, 1992; Dalton and Treisman, 1992; Janknecht *et al.*, 1993; Wasylyk *et al.*, 1990). Furthermore, Ets-1 or v-Ets can activate their target genes, stromelysin and collagenase even in the absence of an *ets*-motif (Wasylyk and Wasylyk, 1992) suggesting that Ets proteins might function as transcriptional modulators (reviewed by Wasylyk *et al.*, 1998).

It has been shown that c-Myb is also one of the transcription factors modulated by Ets. Both wild-type c-Myb and the C-terminus truncated form of Myb cooperate with Ets-2 in the transactivation of the *mim-1* promoter. The cooperation of c-Myb and Ets-2 was specific since Ets-1, Jun, Fos or Myc were unable to show a similar effect (Dudek *et al.*, 1992). In these studies, Ets-2 itself did not activate *mim-1* promoter, even though it did bind to the *mim-1* promoter. While the biological significance of cooperation between Ets-2 and Myb is unclear, recently it was reported that Myb and Ets-2 specifically cooperate in the transactivation of early myeloid gene CD13/APN implicating a role for these two transcription factors in myelopoiesis (Shapiro, 1995). Interestingly, Ets-2 cooperates with A-Myb in transcriptional transactivation assays and enhances its ability to mediate cell proliferation in tissue culture cells (Oh and Reddy, 1997). Interestingly, Ets-2 was unable to cooperate in a similar manner with B-Myb, suggesting that the three members of the *myb* gene family might show differences in their ability to cooperate with other transcription factors (Oh and Reddy, 1998). Domain swapping experiments between c-Myb and B-Myb revealed that Ets-2 failed to cooperate with chimeric molecules that contained the C-terminal domain derived from B-Myb, suggesting that the C-terminus of B-Myb, which acts as a positive regulatory domain in B-*myb* prevents its transcriptional cooperation with Ets-2 (Oh and Reddy, unpublished data). It is interesting to note that Ets-2 has been found to contain a *pointed* domain where ras-signal-dependent phosphorylation occurs, and such ras-

dependent phosphorylation enhances its interaction with other factors (Yang *et al.*, 1996). This could constitute a mechanism by which intracellular signals modulate the transcriptional activity of A-Myb and c-Myb proteins.

### c-EBP/b, NF-M

NF-M was first identified because it activates the expression of the gene encoding cMGF which is a chicken myelomonocyte-specific cytokine (Sterneck *et al.*, 1992). NF-M is specifically expressed in myeloid and macrophage cell lines and binds to *mim-1* promoter. Since the *v-myb* gene of E26 induces the expression of *mim-1* gene only in promyelocytic cells but not in other cell lines expressing v-Myb (Ness *et al.*, 1989), it was suggested that NF-M could be the co-factor required for activation of *mim-1* gene by v-Myb. Further experimentation showed that v-Myb does cooperate with NF-M in the transcriptional activation of *mim-1* promoter. Remarkably, ectopic expression of both *v-myb* and NF-M in erythroid cells and fibroblasts was sufficient to induce endogenous markers of myeloid differentiation, like the *mim-1* and lysozyme genes (Ness *et al.*, 1993). The c-EBP- $\beta$ , which is the murine homologue of NF-M, was also found to cooperate with v-Myb in transactivation of the *mim-1* promoter (Burk *et al.*, 1993). Recent studies have shown that A-Myb and B-Myb also cooperate with NF-M/C-EBP beta in the transactivation of Myb-responsive genes (Foos *et al.*, 1994; Mink *et al.*, 1996) suggesting that all of the Myb family genes are endowed with the ability to cooperate with the tissue-specific transcription factor C/EBP beta.

### CREB binding protein (CBP)/p300

p300 is a nuclear phospho-protein which binds to the adenovirus E1A oncoprotein. It has been suggested that p300 is responsible for driving expression of tissue-specific cellular genes associated with the terminal differentiation state of a cell including P27 WAF1/CIP1 for growth arrest (Hen *et al.*, 1985; Stein and Ziff, 1987; Kitabayashi *et al.*, 1995; Missero *et al.*, 1995). The p300 gene product is a 2414 amino acid protein which contains three separate cysteine/histidine (C/H)-rich regions and a bromodomain interposed between two of them. The most C-terminal C/H region is the E1A binding site (Eckner *et al.*, 1994). Recently CBP, the protein which binds to CREB (cAMP response element-binding protein), has been cloned (Chrivia *et al.*, 1993). The CREB protein is specifically phosphorylated by protein kinase A in response to elevated levels of cAMP (Gonzalez and Montminy, 1989) and activated in its transactivation potential. The CREB transactivation domain is bipartite, consisting of inducible and constitutive activators, termed KID and Q2, respectively, that function synergistically in response to PKA stimulation (Brindle *et al.*, 1993; Quinn, 1993). The KID region binds to N-terminus of CBP and p300 in a Ser-133 phosphorylation-dependent manner (Parker *et al.*, 1996). In addition, it was shown that CBP also

interacts with TF11B, which in turn interacts with TBP. This complex plays a role in the recruitment of RNA polymerase to a promoter, suggesting that CBP may serve as a bridging molecule between transactivators (e.g. phosphorylated CREB) and basal transcription machinery and the RNA polymerase (Kwok *et al.*, 1994) and thereby functions as a co-activator. The amino acid sequence of CBP shows striking homology with p300 in all functional domains. It was shown that CBP and p300 bind to E1A and CREB for the co-activation of CREB suggesting that CBP and p300 represent the same family of the genes (Arany *et al.*, 1995; Lundblad *et al.*, 1995).

It has been shown that c-Myb is another transcription factor which is subject to co-activation by CBP/p300 (Dai *et al.*, 1996). CBP physically associates with the region corresponding to the transactivation domain and the C-terminal flanking region of c-Myb. It also stimulates c-Myb-dependent transactivation, which is blocked by wild-type E1A but not by an E1A deletion mutant lacking the N-terminal region. Since antisense expression of CBP also decreased transactivation by Myb, it was suggested that the c-Myb transactivation is modulated by CBP. However, since c-Myb is a positive regulator of cellular proliferation, the biological significance for the increased Myb transactivation by CBP or p300, which act as promoters of differentiation, remains unclear. Although CBP associates with c-Myb in a phosphorylation-independent manner *in vitro*, it is not known yet whether transcriptional cooperation *in vivo* is phosphorylation-independent. P300/CBP was also shown to physically associate with the transactivation domain and C-terminal flanking region of A-Myb for transcriptional synergy (Dai *et al.*, 1996; Facchinetti *et al.*, 1997; Kiewitz and Wolfes, 1997). It is at present unclear whether P300/CBP binds to B-Myb in a similar manner.

### The cellular target genes of Myb

Several approaches have been used to identify cellular genes activated by Myb. The ts21 E26 virus, which encodes both *myb* and *ets* genes and contains a thermosensitive mutation in the DNA binding domain of Myb was used for the identification of *v-myb* target genes. This conditional mutant, when expressed in chicken myeloid cells at a permissive temperature, was found to induce proliferation of infected cells as immature myeloblasts while at non-permissive temperatures these cells were found to differentiate into macrophage-like cells (Ness, 1989). Using subtractive hybridization, cellular genes which are activated at permissive temperature have been identified. One of the genes was *mim-1*, which codes for a 326 amino acid protein. The promoter region for *mim-1* gene has three Myb binding sites with different affinities for Myb proteins. The *mim-1* gene was expressed at high levels in promyelocytes and at low levels in macrophage cells. The biological function of *mim-1* is not known but it does not appear to be associated with *v-myb* associated transformation of myeloid cells. While the truncated forms of Myb could readily transactivate *mim-1* promoter, c-Myb was found to activate *mim-1* gene only in cooperation with Ets-2 (Dudek *et al.*, 1992) or c-EBP (Burk *et al.*, 1993).

In one study, c-Myb was shown to transactivate the promoter of *c-myb* gene itself in fibroblasts suggesting auto-regulation (Nicolaidis *et al.*, 1991). In contrast, a recent study demonstrates that c-Myb negatively regulates *c-myb* promoter in T-cells (Guerra *et al.*, 1995). The *myb* promoter region was found to contain three Myb binding sites and mutation in the proximal two Myb binding sites markedly increased transactivation of *myb* promoter. However, these Myb binding sites were found to be non-functional in myeloid cells. *c-kit*, which encodes the receptor for stem cell factor was shown to have putative Myb-binding sites, but transactivation of this gene by Myb has not been demonstrated yet (Yamamoto *et al.*, 1993). In T-cells, transfection of c-Myb expression vector was found to increase the level of *c-myc* message and the promoter for *c-myc* was found to be activated by *c-myb* (Evans *et al.*, 1990). Subsequent analysis of *c-myc* promoter showed multiple Myb binding sites around the *myc* promoter P1 and P2 (Nakagoshi *et al.*, 1992; Cogswell *et al.*, 1993). However, it is at present unclear whether c-Myb has a role in the regulation of *c-myc* since in T-cells, induction of *c-myc* transcription by *c-myb* expression plasmid had no effect on the cell cycle progression rate or IL-2 dependence (Evans *et al.*, 1990). Furthermore, *c-myb* is induced in mid to late G1 phase of T-cells after stimulation with IL-2, while *c-myc* is induced in immediate early stage (Reed *et al.*, 1985).

Several surface markers of T-cells were shown to be the targets of Myb. First, the promoter region of *CD4* contains Myb binding sites and Myb binds to the *CD4* promoter. Transactivation by Myb was critical for full promoter function (Siu *et al.*, 1992; Nakayama *et al.*, 1993). In addition, the promoter region of *CD34*, the surface marker for immature and self-renewing cells, contains Myb and Ets-like binding sites (He *et al.*, 1992). It has been shown that c-Myb protein transactivates the *CD34* promoter via specific interaction with multiple Myb binding sites in the 5' flanking region of the gene and induces expression of the endogenous *CD34* mRNA in rodent fibroblasts. Also, constitutive expression of *c-myb* in *CD34*-negative human glioblastoma cells induced expression of *CD34* mRNA and synthesis of the surface membrane antigen (Melloti *et al.*, 1994). In addition, T-cell receptor delta gene was shown to have a Myb binding site and cooperated in transcriptional transactivation with the T-cell-specific core binding factor, CBF/PEBP2 (Hernandez-Munain and Krangel, 1995). Since developmental activation of VDJ recombination in the T cell receptor (TCR) delta locus is controlled by an intronic transcriptional enhancer (E delta) and transcriptional activation by E delta is dependent on c-Myb, it was speculated that c-Myb plays a role in the activation of TCR-delta gene rearrangement. In support of this argument, it was demonstrated that Myb induced transactivation was necessary for activating VDJ recombination of the endogenous TCR-delta locus (Hernandez-Munain *et al.*, 1996).

Two of the cell cycle-related genes were shown to have Myb binding sites in their promoter region. First, the expression of *p34cdc2*, which is required for entrance into mitotic phase, was suggested to be dependent on Myb based on the observation that

inhibition of T-cell activation by antisense *c-myb* oligonucleotide blocked *cdc2* expression at the G1/S boundary (Furukawa *et al.*, 1990). Subsequent analysis of the 5'-flanking region of *cdc2* gene identified two Myb binding sites and transactivation of the gene by c-Myb was demonstrated suggesting that *cdc2* is a target of Myb (Ku *et al.*, 1993). However, in a CTLL-2 cell line and a mouse T-cell line dependent on IL-2, *c-myb* transcript levels remained constitutively high independent of cell cycle and also during IL-2 removal. Since removal of IL-2 down-regulated the expression of *cdc2*, the down-regulation of *cdc2* upon IL-2 removal can not be attributed to the down-regulation of *c-myb* (Dautry *et al.*, 1988). In addition, inhibition of T-cell proliferation with antisense *c-myb* oligonucleotides was associated with down-regulation of DNA polymerase alpha expression (Venturelli *et al.*, 1990). Furthermore, constitutive expression of *c-myb* in thermo-sensitive fibroblasts was found to result in the expression of constitutive levels of DNA polymerase alpha transcript suggesting a functional link between the two genes. However the promoter of DNA polymerase alpha failed to show any transactivation by *myb* despite the presence of Myb binding sequence in the region (Sudo *et al.*, 1992) suggesting that another co-factor or an indirect mechanism could be responsible for this phenomenon. Similarly, constitutive expression of *c-myb* in a thermo-sensitive fibroblast cell line blocked down-regulation of PCNA and Histone H3 transcript levels while parental cells showed no expression of these two genes (Travali *et al.*, 1991). However, nuclear run-on assays demonstrated that the transcription of the two genes are equal in both restrictive and permissive temperatures suggesting that post-transcriptional mechanisms are responsible for the observed differences.

Another approach that was employed for the identification of *myb* target genes was the analysis of differentially expressed genes between *c-myb*<sup>-/-</sup> null mutant and wild-type cells. This approach identified several genes differentially expressed between the two groups. Among these genes, GATA-1, which is a transcription factor binding to the GATA sequence was consistently down-regulated in *c-myb*<sup>-/-</sup> cells (Lin *et al.*, 1996).

In addition to cellular genes, a number of viral genes were found to be responsive for Myb-mediated transactivation. Thus, c-Myb was shown to transactivate AP-1 through interaction with BZLF-1, the Z gene product of EBV. In this case, BZLF was found to transactivate transcription via the AP-1 site only when complexed with c-Myb (Kenney *et al.*, 1992). Myb-mediated transactivation of HIV and HTLV-1 promoters has been reported (Dasgupta *et al.*, 1990, 1992).

In contrast to *c-myb*, relatively few specific target genes have been identified for A-*myb* and B-*myb*. At this point, it is not certain whether the genes activated by *c-myb* are also targeted by B-*myb* or A-*myb*. It is also unclear whether members of this gene family cooperate with each other in the transcriptional transactivation or whether they function as competitive inhibitors of each other. In the case of *mim-1* promoter, expression of B-*myb* has been found to exert an antagonistic effect on v-Myb or c-Myb (Foos *et al.*, 1992).

## Biological function of myb gene family

### Temporal and spatial expression pattern

It is now well established that *c-myb* is predominantly expressed in immature hematopoietic cells (Gonda *et al.*, 1982; Westin *et al.*, 1982; Duprey and Boettiger, 1985). Similarly, *A-myb* is predominantly expressed in the male germ cells (Mettus *et al.*, 1994; Latham *et al.*, 1996; Toscani *et al.*, 1997), although low levels of *A-myb* expression was also detected in ovaries, brain as well as B-cells at the germinal centers (Trauth *et al.*, 1994; Mettus *et al.*, 1994; Foos *et al.*, 1994). In adult male mice, *A-myb* is expressed predominantly in male germ cells. In female mice, *A-myb* is expressed in breast ductal epithelium, mainly during pregnancy-induced ductal branching and alveolar development (Toscani *et al.*, 1997). In contrast to the tissue-specific expression of *A-myb* and *c-myb*, the *B-myb* gene expression seems to be ubiquitous (Nomura *et al.*, 1988). In addition to the tissue-specific expression, developmental expression of these three genes provides additional clues regarding the function of these genes. For example, during development of testis, *B-myb* mRNA is most highly expressed in gonocytes of the fetal testis and spermatogonia and early spermatocytes of the adult. In contrast, *A-myb* mRNA expression begins to increase at post-natal day 10, when primary spermatocytes first appear and in the adult high levels of expression is seen in spermatogonia and primary spermatocytes with concomitant down-regulation of expression in spermatids (Mettus *et al.*, 1994). Thus *B-myb* is expressed primarily in gonocytes and spermatogonia, whereas *A-myb* is required for progression through the first meiotic prophase (Latham *et al.*, 1996). Interestingly, *B-myb* expression during embryogenesis was well correlated with Histone H4, a marker for S phase of cell cycle. However, such coincident expression is not seen during spermatogenesis, suggesting that *B-myb* may perform different functions in cells undergoing mitotic versus meiotic division (Sitzmann *et al.*, 1996). Thus, temporal expression pattern as well as spatial expression pattern of *myb* genes suggest the three *myb* genes perform different biological functions.

### Myb as a regulator of proliferation

To study the role of Myb in cellular proliferation, antisense inhibition of *c-myb* expression has been initially employed. Thus, exposure of normal human bone marrow mononuclear cells to *c-myb* antisense oligodeoxynucleotides resulted in a decrease in both colony size and number, without any apparent effect on the maturation of residual colony cells demonstrating that expression of *c-myb* is critical for hematopoiesis (Gewirtz and Calabretta, 1988). In addition, exposure of peripheral blood mononuclear cells (PBMC) to *c-myb* antisense oligonucleotides blocked T-cell proliferation in response to mitogens with a concomitant block to cell cycle progression in late G1 or early S phase (Gewirtz *et al.*, 1989). Similar effects have been observed with HL-60 and several other myeloid leukemia cell lines where antisense inhibition of *c-myb* blocked their entrance into S phase resulting in a block to their proliferation. These findings

suggested that *c-myb* proto-oncogene is required for maintenance of proliferation and plays a direct role in cell cycle control of hematopoietic cells without affecting their differentiation status (Anofossi *et al.*, 1989; Citro *et al.*, 1992, 1994). However, in another study, *c-myb*-antisense-treated HL-60 cells differentiated only into monocytes but not into granulocytes indicating that granulocytic differentiation, unlike monocytic differentiation, requires *c-myb*-mediated proliferation (Ferrari *et al.*, 1990).

Another approach used to study the biological functions of Myb was through the generation of null mutant mice using targeted disruption of the gene. Homozygous *c-myb* mutant mice produced by gene knock-out techniques developed normally up to day 13 of gestation. However, by day 15, the mutant mice were severely anemic and died in utero (Mucenski *et al.*, 1991). Analysis of these embryos indicated that embryonic erythropoiesis, which occurs in the yolk sac, was not impaired by the absence of *c-myb* expression. However, adult-type erythropoiesis, which first takes place in the fetal liver, was greatly diminished in *c-myb*<sup>-/-</sup> mutants. Other hematopoietic lineages were similarly affected with the exception of megakaryocytes, which showed normal cell number.

Using similar approaches, the role of *B-myb* in the cellular proliferation has also been studied. The anti-*B-myb* oligonucleotides significantly inhibited the proliferation of myeloid or lymphoid cell lines in a dose-dependent manner. This block in proliferation was not accompanied by detectable differentiation of U937 or HL60 cell lines to macrophages or granulocytes either spontaneously or after exposure to chemical agents (Arsura *et al.*, 1992). Similarly antisense inhibition of *B-myb* inhibited proliferation of BALB/c fibroblasts (Sala and Calabretta, 1992), and overexpression of *B-myb* lead to an increase in the number of cells in S phase (Lane *et al.*, 1997; Sala *et al.*, 1996). In addition, constitutive expression of *B-myb* could bypass the Cip-WAF-1 mediated growth arrest in G1 (Lin *et al.*, 1994), suggesting that *B-myb* plays an important role in the transition of cells from G1 into S phase. However, the role of *B-myb* in cell cycle progression was dependent on cell type. For example, quiescent vascular smooth muscle cells express *B-myb* in a cell cycle-dependent manner (Marhamati and Sonenshein, 1996); but quiescent vascular smooth muscle cells were unable to re-enter S phase by co-micro injection of *B-myb* and *c-myc*, whereas co-micro injection of *c-myb* and *c-myc* or *A-myb* and *c-myc* was able to rescue the quiescent cells (Marhamati *et al.*, 1997), implying that specific sub-family of *myb* genes are required for promoting cell cycle progression in specific cell types.

The role of *A-myb* in cell proliferation was initially inferred from its expression pattern in B-cells which correlated with proliferation status; *A-myb* is selectively expressed in germinal centers of spleen, where active cell proliferation occurs, but not in primary follicles, where resting B-cells predominate (Trauth *et al.*, 1994). More direct evidence for *A-myb* induced cell proliferation has been obtained from the observation that co-expression of *A-myb* and *c-myc* leads to re-entrance of quiescent vascular smooth muscle cells into S phase (Marhamati *et al.*, 1997). In another *in vitro* model, overexpression of *A-myb* in

chicken neuroretinal cells led to basic fibroblast growth factor ( $\beta$ -FGF)-dependent proliferation under the condition that mock-transfected cells did not show any growth (Turque *et al.*, 1997), reinforcing the notion that A-myb promotes cell proliferation. Interestingly, in this model, overexpression of A-myb mimicked the phenotype of c-myb overexpressing cells with fibroblast-like appearance and  $\beta$ -FGF-dependent growth. However, unlike c-myb overexpressing cells, A-myb overexpressing cells did not acquire  $\beta$ -FGF-independent growth demonstrating some differences exist between A-myb and c-myb with respect to their ability to promote cell proliferation.

Recently two studies have provided important clues regarding the biological function of A-myb. Transgenic mice expressing constitutively high levels of full length A-myb protein in several tissues were found to develop hyperplasia of the spleen and lymph nodes. These tissues contained a polyclonally expanded B lymphocyte population that expressed a germinal center-cell phenotype. Transgenic B lymphocytes also showed increased DNA synthesis in response to low dose mitogen stimulation, suggesting that A-myb may contribute to hyperplasia by increasing the rate of B cell proliferation (DeRocco *et al.*, 1997). After a 9 month period, these transgenic mice developed abnormalities predominantly in hematopoietic cells, despite the fact that the transgene was expressed in broad ranges of tissues, demonstrating tissue-specificity of the A-myb function.

To determine the role of A-myb in development, we developed homozygous null mutant mice using gene targeting techniques (Toscani *et al.*, 1997). The first observable phenotype of the A-myb<sup>-/-</sup> mice was their small size, apparent during the first few weeks of life. At birth, A-myb<sup>-/-</sup> mice appeared to be indistinguishable from their littermates, but the A-myb<sup>-/-</sup> lagged in growth during the first few weeks after birth. In addition, the A-myb<sup>-/-</sup> pups were small, wrinkled, and exhibited hunched posture as compared to their littermates. As these A-myb<sup>-/-</sup> mice matured (up to 4 months), their small size and hunched posture appearance became less pronounced and these mice attained a body size comparable to the A-myb<sup>+/+</sup> and A-myb<sup>+/-</sup> mice (approximately 90% for the females and 70% for the males). In addition, A-myb<sup>-/-</sup> male mice were found to be sterile and the size and weight of the testes derived from these mice was approximately 25% of their littermates, irrespective of the age. Histopathological analysis of the testes of the A-myb<sup>-/-</sup> mice showed a slight reduction in the size of the seminiferous tubules, though the number of tubules/testis appeared normal. The morphology of the seminiferous tubules in A-myb<sup>-/-</sup> mice was abnormal, with vacuolization of the Sertoli cell cytoplasm and degeneration among the population of primary spermatocytes. While spermatogonia and preleptotene spermatocytes were normal and spermatogonial mitoses were commonly observed, most pachytene primary spermatocytes displayed various levels of degeneration, from early nuclear changes such as increased chromosomal density to advanced stages of cellular breakdown. Most notably, there was a complete absence of post-meiotic cells, such as spermatids or spermatozoa indicating that spermatogenesis in these mice has come to an abrupt halt at the pachytene stage of meiosis.

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The ovaries of the A-myb<sup>-/-</sup> female mice appeared histologically normal. When the A-myb<sup>-/-</sup> female mice were allowed to mate with wild-type of A-myb<sup>+/-</sup> mice, they became pregnant and produced litters of normal number. However, a dramatic abnormality in mammary function became apparent after A-myb<sup>-/-</sup> female mice delivered pups. These female mice were found to be unable to nurse the pups and showed defective mammary tissue proliferation. Histopathological examination of the mammary epithelium revealed that the proliferation of mammary epithelium was considerably diminished in pregnant A-myb<sup>-/-</sup> mice. Specifically, the amount of breast tissue was markedly decreased (by more than 60%) in post-partum A-myb<sup>-/-</sup> mice, when compared to their lactating wild-type or heterozygous littermates. This appeared to be due to reduced cell proliferation resulting in diminished ductal branching following pregnancy. While the breast tissue of post-partum A-myb<sup>-/-</sup> mice appeared to contain a few structures resembling alveoli, they appeared to be incompletely formed. The histopathology of post-partum breast epithelium also showed the presence of considerable amounts of fat tissue in A-myb<sup>-/-</sup> mice, which was almost completely replaced by alveolar structures in A-myb<sup>+/+</sup> or A-myb<sup>+/-</sup> mice. Thus, the loss of A-myb expression seems to result in a loss or diminution of progesterone-induced proliferative events associated with the pregnancy-induced morphogenesis of breast tissue. There appears to be progression toward a differentiated phenotype in the existing ductal tissue, but inadequate proliferation of ductal epithelium seems to result in incomplete and defective alveolar and lobular growth and development. These defects in proliferation could be a result of loss of A-myb which might play a direct role in mediating proliferation of cells, or due to indirect effects caused by alteration of hormone levels which play an important role in breast development.

Although the nature of the A-myb target genes is at present unclear, mRNAs for CREM, Hox1.4, c-abl, and HSP70-2 were totally absent or dramatically decreased in the testes of A-myb<sup>-/-</sup> mice (Toscani *et al.*, 1997). In addition, there was an interesting similarity between A-myb<sup>-/-</sup> and HSP70-2<sup>-/-</sup> mice, in that both mice exhibited an absence of post-meiotic spermatids and mature spermatozoa in their testes. It is also noteworthy that in contrast to c-myb null mutant mice (Lin *et al.*, 1996), GATA-1 transcription was increased rather than decreased in A-myb<sup>-/-</sup> mice (Toscani *et al.*, 1997). Thus A-myb appears to play a critical role in spermatogenesis and mammary gland development and A-myb-mediated gene expression is distinctive from that of c-myb *in vivo*.

### Myb and cell cycle progression

c-myb is highly expressed in immature hematopoietic cells and its expression is down-regulated as they become more differentiated (Westin *et al.*, 1982; Gonda and Metcalf, 1984). In immature cells, c-myb mRNA levels remain high both in proliferating and quiescent cells while in more differentiated cells, the expression of myb is cell cycle-dependent reaching peak levels in mid

to late G1 or S phase. The high levels of *c-myb* transcripts seen in immature cells appear to be due to increased transcription from its promoter while the transient increase seen during cell cycle progression appears to be due to post-transcriptional stabilization of the mRNA (Thompson *et al.*, 1986; Catron *et al.*, 1992). It has been suggested that *B-myb* expression is also cell cycle-regulated since its expression correlates with cell proliferation. Stimulation of quiescent 3T3 fibroblasts with serum was found to result in the induction of *B-myb* expression in late G1 which persisted through the S phase reaching peak levels in the G2/M phase. Repression of *B-myb* expression during G0 and early G1 phases of the cell cycle appears to be mediated by a region in its promoter which was found to contain E2F binding site (Lam and Watson, 1993). Mutagenesis of these E2F consensus binding sites was found to result in an up-regulation of transcription in G0. Furthermore G0 and S phase specific binding of E2F complexes to this element in the *B-myb* promoter were detected in NIH3T3 cell extracts by mobility shift assays, suggesting that the E2F site indeed plays an important role in the regulation of *B-myb* (Lam *et al.*, 1995). Subsequent studies have shown that this element mediates the binding of p107, E2F-4 and DP-1. However, transfection of these genes into cycling NIH3T3 cells did not repress *B-myb* expression suggesting that an additional repression mechanism might be involved (Bennett *et al.*, 1996). In this regard, it is interesting to note that a sequence motif termed CHR (cell cycle genes homology region) exists in the promoters of *B-myb*, cyclin A, *cdc2*, *cdc25C*, all of which are induced in later stages of cell cycle. This CHR region, in conjunction with another major repressor element known as cell cycle-dependent element (CDE) (Zwicker *et al.*, 1998) has been found to mediate the transcriptional repression of these cell cycle genes. A factor that can bind to the CHR element of *B-myb* could be identified in HeLa cell nuclear extracts and it is now believed that both the E2F element and the CHR element work in concert in regulating *B-myb* expression (Liu *et al.*, 1996).

In addition, *B-myb* appears to be phosphorylated during the S phase of the cell cycle (Robinson *et al.*, 1996). It has been suggested that this phosphorylation is mediated by cyclin A or cyclin E, but not by cyclin B. Furthermore, co-expression of cyclin A or cyclin E with *B-myb*, but not *c-myb*, leads to significant increase in *B-myb* transactivation potential implicating a correlation between cyclin-dependent phosphorylation and increase of *B-myb* transactivation potential (Sala *et al.*, 1997). Similar results were also obtained by co-expression of cyclin A, but not cyclin D or cyclin E (Ziebold *et al.*, 1997). Recently it was demonstrated that *B-myb* is a direct target of cyclin A/cdk-2 complex and mutation of cyclin A/Cdk2 phosphorylation sites of B-Myb leads to a significant decrease in the transactivation potential of *B-myb* (Saville and Watson, 1998).

Very similar to cell cycle-dependent expression profile of *B-myb*, *A-myb* expression was also shown to be cell cycle-dependent being induced during S phase. The expression patterns of *A-myb* and *B-myb* in serum stimulated 3T3 cells was overlapping with the induction of *A-myb* RNA occurring slightly later than the *B-myb* message (Ziebold *et al.*, 1997).

Interestingly, A-Myb protein was also shown to be phosphorylated by co-expression of cyclin A or cyclin E, and the co-expression of *A-myb* and cyclin A increased A-Myb transactivation potential (Ziebold and Klemppner, 1997), suggesting that *A-myb* is also regulated in a cell cycle-dependent manner. Surprisingly, however, cyclin A does not appear to be capable of affecting the transactivation potential of *c-myb*, despite the fact that *c-myb* is also regulated in a cell cycle-dependent manner (Ziebold and Klemppner, 1997).

Although both c-Myb and B-Myb exert similar effects in cell cycle progression (Reiss *et al.*, 1991), additional studies have shown that they are independently regulated in cell cycle progression of B cells. Resting tonsillar B cells stimulated with the anti-CD20 antibody alone are induced to enter the G1 but not the S phase of the cell cycle, whereas co-stimulation with the anti-CD40 antibody further drives them to enter the S phase and proliferate. It was shown that while anti-CD40 antibody alone induces *c-myc* mRNA in resting B cells, anti-CD20 antibody induces both *c-myc* and *B-myb*, and the full mitogenic signal given by both antibodies together is accompanied by increased expression of all three genes; *c-myc*, *B-myb*, and *c-myb* (Golay *et al.*, 1992). In addition, several different growth inhibitors caused different modes of down-regulation of these genes suggesting that *c-myb* and *B-myb* are independently regulated and play different roles in regulation of the cell cycle (Golay *et al.*, 1992). Furthermore, in T-cells stimulated with IL-2, *c-myb* transcripts were induced immediately and reached a maximum after 4 h when cells were in early G1 whereas induction of *B-myb* transcription did not reach a maximum until 12 h after stimulation when cells were in S phase suggesting that induction of *c-myb* is a direct response to IL-2 signaling and may be part of the signal transduction while *B-myb* expression is not a direct response to IL-2 signaling but may be related to the intrinsic pathway that controls transition from G1 to S phase (reviewed by Lyon *et al.*, 1994). Thus induction of *B-myb* was later than *c-myb* and showed a greater correlation with the S phase of the cell cycle although *c-myb* is also required for the progression of the cell cycle. Consistent with these findings, ectopic expression of *B-myb* rescued cells from p53-induced G1 arrest even in the presence of WAF/CIP1 transactivation and inhibition of cyclin E/Cdk2 kinase activity suggesting that *B-myb*-induced cell cycle progression bypasses p21CIP1/WAF1-mediated G0/G1 check point. These studies further support the notion that *B-myb* expression is required for transition from G1 into S phase (Lin *et al.*, 1994). Another supporting observation has come from the study on cell cycle-specific phosphorylation of B-Myb. B-Myb was found to be phosphorylated specifically in S phase. The baculovirus purified B-Myb protein was phosphorylated with cyclin A/cdk2, but not with cyclin E/cdk2 or cyclin D1/cdk4 (Robinson *et al.*, 1996). It is not clear yet as to how the signal from *c-myb* and *B-myb* is coordinated for controlled progression of cell cycle or what is the role played by *c-myb* and *B-myb* in this process.

Recently it was shown that the promoter region of *B-myb* contains an E2F binding site which appears to be necessary for E2F-mediated repression of *B-myb* expression during G0/early G1 phase so that this gene

is expressed in G1/S boundary (Lam *et al.*, 1993). This repression was relieved by interaction with p107<sup>Rb</sup> rather than p105<sup>Rb</sup> (Lam and Watson, 1993, 1995). This observation was further supported by recent data demonstrating that the promoter activity is down-regulated by p107 and exogenous overexpression of B-*myb* can increase DNA synthesis in p107 growth-arrested human osteosarcoma cell (SAOS-2) (Sala *et al.*, 1996). In addition, the regulatory B-*myb* promoter site was shown to bind with high affinity to free E2F and to E2F-pocket protein complexes in an indistinguishable manner *in vitro*. In contrast, *in vivo* footprinting with NIH3T3 cells demonstrated that E2F site was occupied specifically in early G1, when the B-*myb* promoter is inactive (Zwicker *et al.*, 1996). Although the *c-myb* promoter region also contains E2F binding site, it is not known yet whether *c-myb* expression is regulated by E2F.

### Myb in differentiation

Since the three members of the *myb* gene family play an important role in cell proliferation, their role in the differentiation process has been a subject of intense investigation. *c-myb* is highly expressed in immature hematopoietic cells and is down-regulated during terminal differentiation of these cells (Westin *et al.*, 1982; Gonda and Metcalf, 1984). Furthermore, cytokine or chemically induced differentiation of myeloid cells or erythroid leukemia cells is accompanied by down-regulation of *c-myb* (Westin *et al.*, 1982; Gonda and Metcalf, 1984; Kuehl *et al.*, 1988; Ramsay *et al.*, 1986). Similarly, differentiation of a neuroblastoma cell line by retinoic acid was also associated with down-regulation of *c-myb* (Thiele *et al.*, 1988). Furthermore, constitutive expression of *c-myb* blocked differentiation of these cell lines (Clarke *et al.*, 1988; McClinton *et al.*, 1990; Todokoro *et al.*, 1988; Selvakumaran *et al.*, 1992; Bies *et al.*, 1995; Patel *et al.*, 1993, 1996). These results suggest that down-regulation of *c-myb* is required for terminal differentiation. Using inducible expression of *c-myb*, it was shown that later phase of down-regulation of *c-myb* is critical for the commitment of cells to terminal differentiation (McClinton *et al.*, 1990; Danish *et al.*, 1992). In addition to the down-regulation of *c-myb* expression during differentiation, transactivation potential of *c-myb* itself was shown to be inhibited by differentiation-inducing agents. Thus in a human monocytic cell line, it was demonstrated that retinoic acid inhibited transactivation of a *myb*-responsive reporter gene without affecting Myb DNA binding activity (Smarda *et al.*, 1995).

Attempts have been made to distinguish the signals required for terminal differentiation and for growth arrest which results from down-regulation of *c-myb*. When the M1 myeloid cell line was treated with differentiation inducers or growth inhibitors, down-regulation of the proto-oncogenes *c-myc* and *c-myb* occurred only when these cells were stimulated to undergo terminal differentiation suggesting that *c-myc* and *c-myb* down-regulation is not necessary for growth suppression (Hoffman-Liebermann and Liebermann, 1991). Furthermore, *v-myb* blocked differentiation but not proliferation in 32D myeloid cells treated with G-

CSF suggesting that G-CSF-induced signal for differentiation is specifically affected by *v-myb* (Patel *et al.*, 1993). In addition there appears to be a lineage specificity in *c-myb*-induced differentiation blockage. In K562 cells, which have potential to differentiate along two lineages, erythroid differentiation by chemicals were selectively blocked by exogenous expression of *c-myb*, while megakaryotic differentiation by TPA was not affected (Rosson and O'Brien, 1995).

Several lines of evidence suggest that B-*myb* and A-*myb* may also play an important role in differentiation. Thus, B-*myb* is expressed in neuroblastoma cell lines and is down-regulated during retinoic acid-induced differentiation. When B-*myb* is constitutively expressed in these cells, the retinoic acid-induced differentiation was blocked suggesting that B-*myb* plays a similar role as *c-myb* in the regulation of differentiation (Raschella *et al.*, 1995). In another study, antisense inhibition of B-Myb expression was unable to rescue the differentiating cells from programmed cell death implicating that B-*myb* function may also be required to block the onset of apoptotic death (Raschella *et al.*, 1995). However, the role of B-*myb* in hematopoietic cell growth and differentiation appears to be very dependent on the nature of stimuli that induce differentiation. For example, in murine M1 cells that are induced to differentiate by interleukin-6 (IL-6), overexpression of B-*myb* prevented growth arrest, which would normally follow terminal differentiation, but with the appearance of differentiation markers (Bies *et al.*, 1996). In contrast, overexpression of B-*myb* in murine 32Dc13 cells accelerated terminal differentiation of cells to a granulocytic lineage with concomitant growth arrest (Oh and Reddy, 1998). In these cells, overexpression of *c-myb* led to continuous growth and blockage to terminal differentiation (Oh and Reddy, 1998). Domain swapping experiments where the various domains of c-Myb were exchanged with those of B-Myb revealed the C-terminal domain of B-*myb* was responsible for the acceleration of terminal differentiation (Oh and Reddy, 1998). Thus the C-terminus of B-Myb, which functions as a positive regulator of transactivation dictates B-Myb specific biological functions in this cell line. During TPA or DMSO-induced differentiation of HL-60 cells, *c-myb* expression levels decrease well before the decrease of B-Myb protein levels and proliferation during differentiation toward monocytic lineage. However, during granulocytic differentiation, *c-myb* levels decreased later than those of B-Myb and proliferation (Arsura *et al.*, 1994).

In contrast to *c-myb* and B-*myb*, little data is available regarding the role of A-*myb* in cellular differentiation. However, it was shown that in testes, where A-*myb* is highly expressed, the abundance of A-*myb* transcript was inversely correlated with maturation status of male germ cells. It would be interesting to see whether overexpression of A-*myb* blocks the ability of germ cells to undergo differentiation and whether *c-myb* and B-*myb* can substitute A-*myb* function. A recent study also shows that A-*myb* is highly expressed only during centoblast stage (CD38 + CD20 + germinal cell) of B-cell development and is rapidly down-regulated during differentiation of these cells to either memory cells or plasma cells (Golay *et al.*, 1998). While A-*myb* is not expressed in myeloid cells, overexpression of full

length A-*myb* in 32Dc13 cell line did not affect the GCSF-induced terminal differentiation of these cells, suggesting that A-*myb* does not function in an identical manner to that of *c-myb* (Oh and Reddy, 1997). However, it is interesting to note that a truncated form of A-*myb*, which lacks the C-terminal negative regulatory domain actively blocks terminal differentiation of 32Dc13 cells, suggesting that the C-terminal domain of A-*myb* determines its functional specificity (Oh and Reddy, 1997).

#### Activation of *myb* gene in murine myeloid tumors

Studies with murine myeloid tumors indicate that *myb* gene is associated with the genesis of some of these tumors induced by leukemia viruses. Thus, it was first noticed by Mushinski *et al.* (1983) that a group of murine tumors, termed ABPL tumors contain a rearranged *c-myb* locus. These tumors arose in BALB/c mice following the injection of pristane and Abelson virus along with its helper, Moloney murine leukemia virus (Potter *et al.*, 1978). In the presence of pristane, which induces intraperitoneal granulomatous tissue, the virus complex rapidly induces a variety of lymphoid neoplasms, predominantly of pre-B cell series, which have been termed Abelson Lymphosarcomas (ABLS tumors). Two other classes of tumors that are induced at much lower frequency by this virus complex are plasmacytomas (ABPC tumors) and a morphological subset of tumors that appear to be myeloid and have been termed ABPL tumors. Later studies indicated that ABPL tumors do not produce Abelson virus nor do they contain the integrated pro-viral genome (Muschinski *et al.*, 1983). Instead, all the ABPL tumors examined were found to have undergone rearrangements in the *myb* locus resulting in the synthesis of abnormal mRNA transcripts. Molecular cloning and structural comparison of the normal and rearranged *c-myb* DNA sequences revealed that the rearrangements in all ABPL tumors were due to the integration of the Mo-MuLV genome into a 1.5 kb stretch of cellular DNA in the third exon of *c-myb* (Shen-Ong *et al.*, 1984, 1986; Lavu and Reddy, 1986). This has been shown to result in the initiation of transcription immediately downstream to the viral integration site which results in the synthesis of mRNA that lacks the 5' *myb*-coding sequences. To understand the nature of the aberrant *myb* transcripts produced in ABPL tumors, Tantravahi *et al.* (1996) isolated the genomic and cDNA clones from ABPL tumor cell lines and carried out their nucleotide sequence analysis. These studies revealed that viral integration in the *myb* locus generates splicing errors at both the 5' and 3' ends. Viral integration results in transcriptional initiation within the LTR sequences and generation of a chimeric RNA that lacks the first three coding exons. At least in one of the ABPL tumor cell line, aberrant splicing events at the 3' end also were found to occur resulting in a truncation of the protein product at the 3' end (Dudek and Reddy, 1989b). The biological significance of this 3' truncation is at present unclear (Tantravahi *et al.*, 1996). Rearrangements in the *c-myb* locus have also been observed in another myeloid tumor line, NFS-60, which was originally induced by Cas-Br Mo-MuLV in NFS mice (Shen-Ong *et al.*, 1986; Weinstein *et al.*, 1986).

Molecular cloning and nucleotide sequence analysis studies demonstrated that the rearrangements are due to the integration of the proviral genome in the *c-myb* locus of this cell line. However, unlike in the case of ABPL tumors, the integration occurs toward the 3' end of the *myb* locus resulting in a premature termination of the rearranged *myb* gene transcription. The *myb* coding sequences in this aberrant mRNA terminate at a point 15 nucleotides upstream to the end of 9th exon. This results in the synthesis of a Myb protein that contains a C-terminal deletion similar to that synthesized in AMV and E26 virus-transformed cells. Following these studies, a number of investigators have examined the rearrangements in *c-myb* locus in chicken and mouse myeloid tumors that result from leukemia virus infection. These studies have identified a number of tumors that contain rearrangements in the *c-myb* locus as a result of retroviral integration. Often, these rearrangements have been found to result from the integration of retroviral genome in the first, second or third exons resulting in the deletion of coding sequences derived from exons 1, 2 and/or 3. Integration of a retroviral genome in these introns results in the deregulation of *c-myb* expression as well as truncation of its N-terminal sequences (reviewed by Wolff, 1996). It is possible that both these events, enhanced levels of Myb protein expression and truncation of its N-terminal end contribute to the oncogenic activity of the protein.

#### Activation of the *myb* gene in human tumors

The human *myb* gene is located on chromosome 6Q22-24 (Zabel *et al.*, 1984) and abnormalities in this locus have been observed in several acute myelogenous leukemias, T-cell leukemias, colon carcinomas as well as several melanomas (Alitalo *et al.*, 1984; Barletta *et al.*, 1987; Pellici *et al.*, 1984; Dasgupta and Reddy, 1989). In a majority of these tumors, these abnormalities seem to be accompanied by an amplification of the *myb* gene followed by enhanced transcription. Rearrangements have not been generally observed in these tumors. However, it is possible that amplification could result in point mutations or splicing aberrations that result in the structural alterations of the protein synthesized. This aspect needs to be further investigated. More recently, our laboratory (Dasgupta *et al.*, 1989) has analysed *myb* rearrangements in a melanoma with a translocation in the 6Q22 region. These studies indicate that the breakpoint in these tumors occurs in the carboxy-terminal region of the *myb*-coding region, a situation similar to that observed with murine tumors. Interestingly, the rearrangement is accompanied by transcriptional activation in the metastatic forms of this tumor, implicating this translocation in tumor progression.

More recent studies have shown the existence of a positive correlation between the expression of the estrogen receptor (ER) and *c-myb* in primary breast tumors and cell lines which are derived from these tumors (Guerin *et al.*, 1990). ER-positive breast tumor cell lines also show *c-myb* expression, while those breast carcinoma cell lines which are ER-negative fail to express *c-myb* RNA. As there is a positive correlation between the status of the ER and *c-myb*

in primary tumor cell lines, it has been proposed that estrogens either directly or indirectly influence the expression of the *c-myb* gene. This correlation between the expression of *c-myb* and the ER is indicative of a role for *c-myb* during normal breast development. Our *in situ* hybridization studies also show that *c-myb* expression positively correlates with the proliferation and ductal branching of normal breast epithelium (our unpublished data) during the adolescent stages of development, which is mediated by estrogens. Thus, during normal development, *c-myb*, like estrogen, may be required as a regulator of proliferative events which result in the expansion and early refinement of the ductal system. In addition, an ensuing inability to down-regulate *c-myb* in a timely fashion may represent a point at which cells within the mammary gland acquire oncogenic potential.

A similar correlation between *A-myb* and the progesterone receptor can be deduced. The ductal networks undergo additional branching and specialization in response to progesterone which is supplied by

the placenta during pregnancy. There is a striking similarity in the phenotypes of female *PR<sup>-/-</sup>* and *A-myb<sup>-/-</sup>* mice. Although the mammary glands of these mice develop normally during embryogenesis and adolescence, they fail to significantly undergo secondary proliferative events and generate alveolar cells which have the capacity to synthesize and secrete milk (Toscani *et al.*, 1997). Thus, like *c-myb*, *A-myb* appears to be required for the transition between distinct stages of breast development and the subsequent proliferation events which are necessary for ductal growth and morphogenesis. We have recently determined that ER-negative breast carcinoma cell lines express high levels of the *A-myb* mRNA. These results suggest that an inability to down-regulate *A-myb* expression following pregnancy-induced differentiation may either represent a point at which the onset of breast cancer can occur, or an oncogenic event, which in combination with other mutations, compounds and facilitates the abrogation of hormone responsiveness.

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