The DNA Binding Domain of the A-MYB Transcription Factor Is Responsible for Its B Cell-specific Activity and Binds to a B Cell 110-kDa Nuclear Protein*

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Expression studies as well as the use of transgenic animals have demonstrated that the A-MYB transcription factor plays central and specific role in the regulation of mature B cell proliferation and/or differentiation. Furthermore, it is highly expressed in Burkitt’s lymphoma cells and may participate in the pathogenesis of this disease. We have therefore investigated the transcriptional activity of A-MYB and its regulation in several human lymphoid cell lines using co-transfection assays and show that A-MYB is transcriptionally active in all the B cell lines studied, but not in T cells. In particular the best responder cell line was the Burkitt’s cell line Namalwa. The activity of A-MYB in B and not T cells was observed when either an artificial construct or the c-MYC promoter was used as a reporter. Furthermore, the functional domains responsible for DNA binding, transactivation, and negative regulation, previously characterized in a fibroblast context, were found to have similar activity in B cells. The region of A-MYB responsible for the B cell specific activity was defined to be the N-terminal 218 amino acids containing the DNA binding domain. Finally, a 110-kDa protein has been identified in the nuclei of all the B, but not T, cell lines that specifically binds to this A-MYB N-terminal domain. We hypothesize that this 110-kDa protein may be a functionally important B cell-specific co-activator of A-MYB.

Transcription factors control crucial cellular processes such as cell growth and differentiation. Deregeneration of their function or of their expression has been implicated in many hematopoietic neoplasms (1). A-MYB is a member of the Myb family of transcription factors (2–4). All members of the Myb family have been implicated directly in the control of cell growth and differentiation (reviewed in Refs. 3 and 4); the v-MYB oncogene transforms cells of hematopoietic origin (5), its normal cellular equivalent c-MYB is required for hemopoiesis (6) and B-MYB is involved in the regulation of proliferation and differentiation of many lineages (7–9). Finally A-MYB plays a role in restricted lineages and during a specific stage of their differentiation. Among hematopoietic cells, A-MYB expression is restricted to a narrow window of normal B cell differentiation, taking place in germinal centers (10, 11). Furthermore it is highly expressed in Burkitt’s lymphoma (BL) cells and not in most other neoplastic B cells (12). Very recently, transgenic mice expressing high levels of ectopic A-MYB in most tissues have been found to show follicular hyperplasia and enhanced B cell proliferation, demonstrating that A-MYB plays an important and specific role in regulating B cell proliferation and/or differentiation (13). These data as well as its ability to cooperate with c-MYC in promoting entry into S phase (14) strongly suggest that it is an important factor in the pathogenesis of BL (12, 13). A-MYB is expressed in few other nonhematopoietic tissues: spermatocytes, some epithelial cells, and some central nervous system cells (2, 11, 15). A-MYB knockout mice show defects in spermatogenesis and mammary gland formation in agreement with the normal expression pattern of the gene (16).

The transcriptional activity of A-MYB and its regulation have never previously been investigated in B lymphocytes, one of the cellular context in which it plays an important function. Several functional and regulatory domains of human A-MYB have been recently characterized in murine fibroblasts (17, 18). Amino acids 33–188 contain the three imperfect repeats of 51–52 aa, which form the DNA binding domain (19). The transactivation domain is formed by about 100 aa centered around a stretch of charged amino acids (aa 218–318). The C-terminal half of the molecule is inhibitory for both DNA binding and transactivation (17, 18). In this report we have set out to investigate the activity of A-MYB in B cells. We show that the A-MYB transcriptional activity can be detected in all B cell lines studied, being the highest in BL cells. By contrast A-MYB was not active in the T cell lines. Furthermore we have defined the DNA binding domain of A-MYB as being responsible for its B cell specific activity and have identified a 110-kDa protein present in B, but not T, cells that is able to bind to the A-MYB DNA binding domain.

EXPERIMENTAL PROCEDURES

Cell Lines and Cell Cultures—The Burkitt’s lymphoma cell line Namalwa was a kind gift of Prof. D. H. Crawford (London School of Hygiene and Tropical Medicine, London). The myeloma cell line IM-9, the acute lymphoblastic T leukemia line CEM, and the T cell leukemia line Jurkat were from the American Tissue Culture Collection (ATCC; Rockville, MD). The BJAB non-Hodgkin lymphoma line was a gift from Dr. Vercelli, Dibit Center, Ospedale San Raffaele, Milan, Italy. All lines were grown in RPMI 1640 medium (Seromed, Berlin) supplemented with 10% fetal calf serum (HyClone, Steril System, Logan, UT), glutamine (Life Technologies, Inc., Paisley, Scotland), and 50 µg/ml hygromycin B.

1 The abbreviations used are: BL, Burkitt’s lymphoma; aa, amino acids; CAT, chloramphenicol acetyltransferase; wt, wild type.
gentamycin (Life Technologies, Inc.).

**Transient Transfections and CAT Assays**—Cells were resuspended in fresh medium at a density of $2 \times 10^5$ cells/ml 24 h prior to transfection. For transfections, $5 \times 10^4$ cells were incubated for 5–50 min at 37°C in 1 ml of transfection medium (culture medium lacking fetal calf serum and containing 50 mg/ml tryps-in HCl (pH 7.4), containing 150–500 μg/ml DEAE-Dextran (Pharmacia Biotech Inc., Uppsala, Sweden) and 15 μg of plasmid DNA (3 μg of β-galactosidase plasmid pON405 as standard, 3 μg of CAT reporter plasmid, and the indicated quantities of the A-MYB expression plasmids and/or empty expression vector). The transfection conditions were optimized for each cell line. The cells were then washed and cultured in 5 ml of complete medium for 48 h. The harvested cells were lysed by repeated freezing and thawing, and the lysates were cleared by centrifugation at 13,000 rpm for 30 min. Equal amounts of total protein, determined with the Bio-Rad protein assay (Bio-Rad), were used for β-galactosidase and CAT assays. CAT assays were performed using a phase-extraction protocol (20). The CAT activity values were normalized to the β-galactosidase activity.

**Plasmid Constructions**—The KHK-CAT plasmid was as described (21). The A-MYB wt, pCAD1, pΔ1, and mutant 1 expression constructs have been described previously (19) and are all subcloned in the pSG5 expression vector containing the SV40 promoter (Stratagene). The MYC promoter CAT construct was a kind gift of Dr. L. Lombardi (Institute of Medical Sciences, Ospedale Maggiore, Milan, Italy) and contains the human MYC promoter from the HindIII to SV40 downstream dimer site 2.5 kilobases upstream from the first exon of c-MYC, to the PvuII site at the end of the first exon, placed upstream from the CAT gene in pSVOCAT. The GAL4 reporter construct 17MX2-He-CAT contains two copies of the GAL4 binding site cloned upstream from the CAT gene (22). The β-galactosidase plasmid pON405 contains the β-galactosidase cDNA cloned downstream from the cytomegalovirus promoter. pGA was generated by inserting the blunt NcoI-EcoRV fragment from A-MYB mutant 1 (17) in the blunted ClaI site of the pG4 poly II plasmid carrying part of the GAL4 cDNA (23). The construct encodes a hybrid protein consisting of the GAL4 DNA binding domain from aa 1 to 148 fused in frame to the A-MYB transactivation domain from aa 218 to 318. The Gal4 DNA binding domain alone (pG-) was encoded by the pG4 poly II plasmid (23). pGA was made by first inserting the XhoI fragment of VP16 into the NcoI site of the A-MYB cDNA cloned in the pECE vector and then transferring the complete insert into the BamHI site of the pSG5 vector (Stratagene), resulting in a hybrid containing the A-MYB DNA binding domain (aa 1–217) fused in frame to the transactivation domain of VP16 (aa 411–490). The pG7 control plasmid carried the same XhoI fragment of VP16 fused in frame to the Gal4 DNA binding domain encoding aa 1–148. A control plasmid carrying only the complete A-MYB DNA binding domain (pΔ-) in pSG5 was made by carboxy truncation at a mutated BamHI site at aa position 262 (17). The pGST-A-MYB-BDB construct was made by cloning the 650-base pair NcoI fragment from p1500 into the EcoRI site of pGEX-3X (Pharmacia). This construct encodes the glutathione transferase protein in frame with the A-MYB DNA binding domain (aa 2–217) as constructed by sequencing.

**Recombinant Protein Production and Purification**—Bacteria strain K803 was used for producing the GST and GST-A-MYB-BDB fusion proteins. After induction for 3 h with 0.1 mM isopropyl-β-D-thiogalactopyranoside the bacteria were harvested and resuspended in Solution A (50 mM HEPES, pH 8.0, 150 mM NaCl, 5 mM KCl, 1 mM dithiothreitol, 1 mM EDTA, 10% glycerol) plus 0.1 mg/ml lysozyme and incubated at room temperature for 10 min. The cells were treated then by several cycles of sonication on ice, and the lysates containing the soluble recombinant protein were obtained by centrifugation. The lysates were incubated with glutathione-agarose beads (Sigma) for 1.5 h at 4°C, washed three times with Solution A, and either eluted with glutathione or used directly for protein binding assays.

**Metabolic Labeling and Protein Binding Assay**—$1 \times 10^{10}$ exponentially growing cells were cultured for 3 h in methionine- and cysteine-free RPMI medium containing 200 μCi of pro-Mix (Amersham) cell labeling mix (1000 Ci/mmol, Amersham). Labeled cells were lysed in 200 μl of Buffer 1 (20 mM HEPES, pH 8.0, 25 mM KCl, 5 mM MgCl₂, 0.5% Nonidet P-40, 5 mM dithiothreitol, protease inhibitors) and centrifuged at 1500 rpm for 5 min at 4°C. The supernatant contained the cytoplasmic fraction, and the nuclear pellet was further extracted for 10 min at 4°C in 100 μl of Buffer 2 (20 mM HEPES, pH 8.0, 1 mM EDTA, 5 mM MgCl₂, 0.5% NaCl, 20% glycerol, protease inhibitors) and cleared by centrifugation. Both cytoplasmic and nuclear extracts were diluted 1:4 in Buffer 3 (20 mM HEPES, pH 8.0, 1 mM EDTA, 5 mM MgCl₂, 20% glycerol, protease inhibitors) and incubated with 20 μl of GST-bound glutathione agarose for 1.5 h at 4°C and centrifuged. These precleared extracts were further incubated 1.5 h at 4°C with 20 μl of GST-A-MYB-BDB-bound glutathione agarose. The agarose beads were washed three times with Buffer 4 (20 mM HEPES, pH 8.0, 1 mM EDTA, 5 mM MgCl₂, 100 mM NaCl, 0.02% Nonidet P-40), analyzed on a 8% SDS-polyacrylamide gel, and detected by autoradiography.

### RESULTS

**A-MYB Is Active in B Cells but Not in T Cells**—Previously we had characterized the transcriptional activity and several functional domains of the A-MYB protein in transfected mouse 3T3 fibroblasts (17, 19). Since A-MYB is expressed and shows biological activity in vivo during a narrow stage of B lymphoid differentiation and not in other hematopoietic lineages (10, 12, 18), we set out to more directly investigate the transcriptional activity and functional domains of A-MYB in human hematopoietic cells. For this purpose, we used the wt A-MYB cDNA, a C-terminal truncated mutant (mutant 1), previously shown to be 5–6-fold more active than the wild type in fibroblasts (17), as well as two mutants carrying deletions of the previously defined DNA binding and transactivation domains (pCAD1 and pΔ1, respectively) (19) (Fig. 1). All constructs were cloned in the pSG5 vector carrying the SV40 promoter and expressed comparable levels of nuclear A-MYB protein after transfection in NIH-3T3 cells (17, 19).

The transient transfection protocols were optimized individually for each of a number of hematopoietic cell lines using the DEAE-Dextran technique. Protocols were found which led to comparable levels of transfection efficiencies in different human T and B cell lines, as measured using both the β-galactosidase and Rous sarcoma virus-CAT reporters (data not shown and Table 1). On the other hand, the maximal transfection efficiencies obtained with several myeloid and erythroid cell lines were at least 10 times lower than those obtained with lymphoid cells (data not shown). Studies on the A-MYB activity were therefore performed only with the more efficient lymphoid cell lines. Fig. 2 (upper panel) shows one representative dose

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**Fig. 1. Schematic representation of the A-MYB wt, mutant, and hybrid constructs**. The structure of the wt A-MYB protein is indicated on top, with the location of the functional domains previously defined in fibroblasts. The three arrows in the DNA binding domain refer to the three tandem repeats. The numbers below each construct indicate the first and last amino acid of the A-MYB fragments present in each protein. All constructs were cloned in the pSG5 expression vector.
The transcriptional activity of A-MYB and mutants in different cell lines

| Cell line      | Cell type | Mean transactivation fold (S.D.)   | % acylation (S.D.)   |
|----------------|-----------|-----------------------------------|---------------------|
|                |           | A-MYB wt<sup>a</sup> | A-MYB wt<sup>a</sup>       | pAV    | pGA    |
| Namalwa        | B (BL)    | 31 (5) | 52 (4) | 13 (2) | 37 (13) | 15 (3) | 12 (1) | 38 (13) |
| BJAB           | B (NHL)   | 1.3 (0.2) | 21 (1) | ND    | ND    | ND    | ND    | 59 (8) |
| IM-9           | B (myeloma)| 1.6 (0.3) | 20 (1) | ND    | ND    | ND    | ND    | 43 (3) |
| EBV            | B (EBV-LCL)| 0.8 (0.1) | 19 (3) | ND    | ND    | ND    | ND    | 10 (4) |
| Jurkat         | T (T leukemia) | 1.5 (0.4) | 1.2 (0.7) | 1.0 (0.2) | 1.3 (0.3) | 1.7 (0.2) | 18 (9) | 53 (23) |
| CEM            | T (T-ALL) | 1.1 (0.3) | 1.3 (0.2) | ND    | ND    | ND    | ND    | 39 (18) |

<sup>a</sup> The data are the mean and S.D. (values in parentheses) of at least three values obtained by transfecting 9 μg of each A-MYB construct. Values above 2 are shown in bold.

<sup>b</sup> The mean percent acylation levels and S.D. (values in parentheses) obtained after transfection of 3 μg of RSV-CAT plasmid are shown.

<sup>c</sup> The A-MYB construct is indicated and below the reporter used.

FIG. 2. Transcriptional activity of A-MYB wt and mutant 1 in lymphoid cell lines. The indicated cell lines were transiently transfected with the KHK-CAT reporter, which carries eight Myb binding sites upstream from the CAT gene. Table I shows the mean activity and standard deviation of several experiments performed with the highest dose of each construct (9 μg), as well as a measure of the transfection efficiencies obtained for the different cell lines with a constant amount of the Rous sarcoma virus-CAT plasmid. Out of six different lymphoid cell lines tested (four B cell and two T cell lines), wild type A-MYB was significantly active only in the B cell line Namalwa, with an up to 40-fold induction of transcription (Fig. 2, upper panel, and Table I). The bell-shaped curve evaluable in the dose-response experiment has already been observed in 3T3 cells and is presumably due to a squelching effect (18, 19). The C-terminally truncated mutant 1, on the other hand, reproducibly showed transcriptional activity in all B cell lines tested, with a 19–21-fold maximal induction in most cell lines and a stronger activity (up to 55-fold) in Namalwa cells (Fig. 2, lower panel, and Table I). On the contrary, mutant 1, like wt A-MYB, did not show any transcriptional activity in the two T cell lines tested and in repeated experiments (Fig. 2 and Table I). Thus the Namalwa cell line (a BL) is the best responder for A-MYB activity. Mutant 1 is more active than wild type in B cells as previously observed in NIH-3T3 fibroblasts, but its activity is cell type-specific, being detected only in B cell lines representative of different stages of differentiation (BL, NHL, myeloma, and EBV-LCL) but not in the two T cell lines studied (one derived from an acute T-lymphoblastic leukemia and the other from a T cell leukemia).

To verify whether the specificity of action of A-MYB in B versus T cells can be observed also when using a normal biologically relevant promoter rather than an artificial construct, we have used the c-MYC promoter, which has been shown previously to be induced by A-MYB (14). As shown in Fig. 3 and Table I, A-MYB wt can also activate the human c-MYC promoter by at least 10-fold in Namalwa cells. As expected, the mutant 1 was more active than wild type on the MYC promoter in the same cells with up to 55-fold activation (Fig. 3 and Table I). That this activity was dependent on both the DNA binding and transactivation domains of A-MYB was verified using the pAV and pCAD1 constructs, which completely lost activity in Namalwa cells (Fig. 3). Interestingly, neither the wt A-MYB protein nor the mutant 1 proteins showed any activity in Jurkat cells using the c-MYC promoter construct (Fig. 3 and Table I). As expected the deletion mutants pAV and pCAD1 were also inactive (Fig. 3). Thus we conclude that i) A-MYB is able to activate efficiently the c-MYC promoter in a B cell context, ii) the A-MYB functional domains previously identified in fibroblasts show similar functions in a B cell context and using a natural and biologically relevant promoter, and iii) A-MYB is completely inactive in T cells even when using such promoter.

The A-MYB DNA Binding Domain Is Responsible for Its Specific Activity in B Cells—The shortest mutant 1 protein contains only the DNA binding and transactivation domains of A-MYB (aa 1–319, Fig. 1) and shows a striking difference in activity in T cells versus B cells using different promoters. We therefore wanted to further define which of these two domains
may be responsible for this difference in activity. For this purpose we constructed two additional mutants, one (pAV) containing the A-MYB DNA binding domain only, fused in frame with the VP16 transactivation domain, and the other (pGA) containing the GAL4 DNA binding domain fused in frame to the A-MYB transactivation domain. As controls, we also used a hybrid construct (pGV) carrying the Gal4 DNA binding domain fused to the VP16 transactivation domain, as well as the DNA binding domains alone (pG- and pA-). All constructs are described schematically in Fig. 1. The hybrids were tested in parallel in the Namalwa and Jurkat cell lines using the KHK-CAT reporter or a GAL4 reporter, as appropriate. As shown in Fig. 4 and Table I, all constructs carrying both a DNA binding and transactivation domain (pAV, pGA, and pGV) were active in Namalwa cells (in a dose-dependent manner). On the other hand, only the pGA and pGV hybrids, but not pAV, functioned in Jurkat cells. As expected, the control DNA binding domains alone (pG- and pA-) were inactive in both cell lines against their respective promoters (Fig. 4). Thus both the A-MYB and VP16 transactivation domains are functional in Jurkat, when fused to the GAL4 DNA binding domain. On the contrary, the A-MYB DNA binding domain fused to the strong VP16 transactivation domain is still inactive in T cells, whereas this fusion construct is active in Namalwa cells (Fig. 4 and Table I).

The A-MYB DNA Binding Domain Binds a 110-kDa Protein Specifically Present in B Cells—The above data suggested that either a co-activator is present in B and not T lymphoid cells or that an inhibitor is present in T and not B lymphoid cells and that these putative co-activator(s) or inhibitor(s) act through the DNA binding domain of A-MYB. To identify such putative co-activator or inhibitor, we subcloned the A-MYB DNA binding domain fused in frame with the GST protein in a bacterial expression vector. Expression of this construct (called GST-A-MYB-DBD) was induced in bacteria and the recombinant protein purified on a glutathione column as shown in Fig. 5A. Finally, metabolically labeled lymphoid cells were lysed, and both the cytosolic and nuclear lysates were passed over the column. As shown in Fig. 5B, one labeled band of approximately 110 kDa (indicated with an arrow) which specifically binds the A-MYB-DBD is observed in the nuclear lysates of all B cell lines tested (Namalwa, IM-9, and EBV-LCL cells in lanes 8, 12, and 14, respectively) but is not detected in the two T cell lines (Jurkat and CEM, lanes 7 and 10). The 110-kDa B cell protein is mostly nuclear and does not bind to GST alone (lane 4). Several other bands appear to specifically bind to the A-MYB DNA binding domain, two of which localize to the cytoplasm and two in the nucleus. These were, however, detected in all lymphoid extracts tested. The same results have been observed in at least three separate experiments.

We conclude that B cells contain a nuclear protein of about 110 kDa, which specifically binds to the A-MYB DNA binding domain, but is not present in T cells. This protein may be responsible for the activity of A-MYB in B versus T cells.

**DISCUSSION**

In this report we show that the A-MYB transcription factor is functionally active in human lymphoid B cell but not in T cell lines. The functional domains previously characterized in fibroblasts (17, 19), in particular the DNA binding, transactivation, and C-terminal negative regulatory domains, appear to have the same function in B cells and also when a natural promoter such as the c-MYC promoter is used as a reporter. We show that the region responsible for the specificity of activity of A-MYB in B versus T cells is the N-terminal region, which includes the DNA binding domain. Finally, in an attempt to characterize the molecular reasons for this cellular specificity, we have identified a 110-kDa protein present in the nuclei of all
Our finding that A-MYB is active in B, but not T, cells is of particular interest in light of the recent report using A-MYB transgenic mice and showing that A-MYB has a strong and specific functional effect on B lymphocyte proliferation and/or differentiation (13). Although these mice expressed the transgene in many tissues, abnormalities were confined primarily to B lymphocytes. Hyperplasia of spleen and lymph nodes were mostly due to an expanded B cell population showing a germinal center phenotype (13). These data are in line with previous work showing that, among hematopoietic cells, expression of the endogenous A-MYB gene is restricted to the activated B cells present in germinal centers (10, 11). Furthermore A-MYB expression is also restricted in B cell neoplasms, being found only in BL and some chronic lymphocytic leukemias (12). These data, together, show that A-MYB plays an important functional role during activation and/or differentiation of mature B lymphocytes. The activity of the Myb family of genes has nearly always been studied in fibroblasts or epithelial cells which are most easily transfected for transient assays (reviewed in Refs. 3 and 25). The only other cell type that has been used to study Myb transcriptional activity is the chicken macrophage line HD11 (11, 26). Thus this is the first report of the study of the activity of a Myb transcription factor in human hematopoietic cells. Our data show that A-MYB is transcriptionally active in all B cell lines studied and not in T cells. The cellular specificity of A-MYB transcriptional activity in B versus T cells is of particular interest. We believe that the difference reflected a true biological property of A-MYB, since it was observed in all the lymphoid cell lines tested, all of which showed comparable transfection efficiencies. The activity of the A-MYB wt was detectable only in Namalwa cells, which were the best responder cell line also for the stronger mutant 1. Interestingly, the Namalwa cell line is derived from a BL patient and A-MYB is highly expressed in most BL cell lines, although the Namalwa line itself does not express significant endogenous A-MYB (12). Furthermore the phenotype of BL cells corresponds to that of normal germinal center B cells, which are the cells that accumulate in the A-MYB transgenic animals. Activity of the stronger mutant 1 could be detected in all B cell lines, but not in T cells. This difference could not be due to the levels of expression of the endogenous MYB genes since we found no correlation between A-MYB activity and levels of A-MYB, B-MYB, or c-MYB mRNA or protein in the same cell lines (12). For this reason, we have started a molecular characterization of the tissue specificity of the A-MYB gene. In this report we show that the presence of the first 217 N-terminal amino acids that contains the DNA binding domain is sufficient to give B cell-specific activity when fused to the VP16 transactivation domain. On the other hand, the A-MYB transactivation domain (aa 218–318) is functional in both T and B cells when fused to the GAL4 DNA binding domain. Furthermore, we show in binding assays using bacterially produced GST-A-MYB that all B cell lines tested, but not T cells, contain a nuclear protein of about 110 kDa, which specifically binds to the A-MYB DNA binding domain. On the basis of these results we hypothesize that this 110-kDa nuclear protein found in B cells is a co-activator of A-MYB function. We do not know the molecular basis for the higher A-MYB activity in Namalwa relative to other B cells, since equivalent amounts of p110 were detected in all B cell lines; thus this difference may depend on other factors binding to A-MYB (17). On the other hand p110 may be responsible, at least in part, for the specific functional effects of the A-MYB transgene in B cells. The confirmation of such hypotheses will require the cloning of this 110-kDa protein, which is under way and is beyond the scope of this paper. Recently a 100-kDa protein called p100 has been described that binds to the DNA binding domain of c-MYB (27). We have evidence that our 110-kDa protein is not p100, since p110 did not react in Western blots with an anti-p100 antiserum (kindly provided by Dr. E. Kieff, Harvard University, Boston). Furthermore p100 has been reported to be ubiquitous (27, 28), and its role in regulating c-MYB transcriptional activity is unclear at present (27).

It is intriguing that the domain responsible for cell type-specific activity is the DNA binding domain. We do not know whether the lack of transactivation activity of A-MYB in T cells reflects lack of DNA binding. The transfection efficiency was too low in either T or B cells to measure DNA binding activity directly. We therefore investigated whether preincubation of...
A-MYB-transfected 3T3 fibroblast extracts with either T or B cell nuclear extracts affected A-MYB DNA binding. We did not observe any measurable effect of the extracts on binding. A more detailed understanding of the mechanism of regulation of A-MYB activity will therefore require cloning of the putative co-factors such as p110 involved in this regulation. This report also shows that the functional domains of A-MYB previously characterized in fibroblasts have the same properties in B cells (17). In particular the transactivation domain of A-MYB has been shown here to be functional also when fused to a heterologous DNA binding domain (from GAL4). In addition the C-terminal half of the protein shows strong inhibitory activity, reducing transactivation by 2–3-fold in a similar manner to what had been observed in NIH-3T3 cells (17). Furthermore we show here that this effect is apparent also when a natural and biologically relevant promoter is being used as a reporter, the human c-MYC promoter. Previously the murine c-MYC promoter had been shown to be regulated by A-MYB in smooth muscle cells (14). Interestingly the c-MYC gene is expressed in the centroblasts of germinal center, like A-MYB (29), and more importantly is the target of chromosomal translocations in 100% Burkitt's lymphomas; for this reason it is considered as a crucial element in the transformation of such tumors (30). One possible speculation is that A-MYB and c-MYC may amplify each other and cooperate in the oncogenic effect (14).

In conclusion, the A-MYB transcription factor is expressed and is functional during a narrow stage of B cell lymphoid differentiation and may function in this context by cooperating with B cell-specific co-factors. This information is reminiscent of what has been previously observed for other transcription factors, which can acquire B cell specificity through interaction with co-factors (31–33), and supports the notion that A-MYB plays an important role in the B cell differentiation process itself.

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