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Antibodies to the evolutionarily conserved amino-terminal region of the v-*myb*-encoded protein detect the c-*myb* protein in widely divergent metazoan species

(*myb* oncogene/conserved protein domain/anti-*myb*-protein antiserum)

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ABSTRACT **Antibodies directed against a bacterial fusion protein that contains the domain encoded by the highly evolutionarily conserved 5′ one-third of the v-*myb* oncogene of avian myeloblastosis virus (AMV) detect the protein products of various members of the *myb* gene family. Immunoprecipitation or immunoblot analyses with these antibodies yielded the following information. First, the products of the v-*myb* oncogenes of AMV ($p48^{v\text{-}myb}$) and of E26 virus ($p135^{gag\text{-}myb\text{-}ets}$) contain this highly conserved amino acid sequence, as previously hypothesized. Second, $p75^{c\text{-}myb}$, the product of the chicken c-*myb* protooncogene, also contains this protein domain. Third, these antibodies have identified the products of the human, murine, and *Drosophila* c-*myb* genes, which were all found to be nuclear proteins of M_r 75,000–80,000. The human c-*myb* protein product is present in immature cells of the erythroid, myeloid, and lymphoid lineages.**

The known oncogenic members of the *myb* gene family are the closely related retroviral oncogenes of the avian myeloblastosis virus (AMV), v-myb^{AMV}, and of the avian E26 leukemia virus, v-myb^{E26} (1–3). AMV causes acute myeloblastic leukemia in chickens and transforms chicken myeloid cells *in vitro* (4). E26 virus, which contains a second oncogene, *ets*, in addition to *myb* sequences, transforms both myeloid and erythroid cells in chickens and *in vitro* (5). The two *myb* viral oncogenes have resulted from two independent transductions of DNA sequences from the single-copy chicken c-*myb* protooncogene (5–8). As is the case for numerous other protooncogenes, c-*myb* has been highly conserved during evolution (9–11).

The v-myb^{AMV} product, $p48^{v\text{-}myb}$ (Fig. 1), was first identified immunologically in virally transformed cells by antisera raised against synthetic peptides deduced from the v-myb^{AMV} DNA sequence (14) or by antiserum against the product of a v-myb^{AMV} sequence fused to a bacterial expression vector (16). All of these antibodies were directed against sequences encoded downstream of the first *myb*-specific ATG (6, 7). The molecular weight of this nonglycosylated protein, 48,000, greatly exceeded that of the predicted M_r 30,000 protein that would have resulted from translation initiation at the first internal AUG of v-myb^{AMV} (14, 16). This led to the hypothesis that translation of the v-myb^{AMV} gene product begins in the spliced *gag* leader sequence, as is the case for the avian retroviral *env* gene product (17). Consistent with this hypothesis, nuclease S1 mapping studies of viral subgenomic mRNA identified a v-myb^{AMV} splice acceptor site that would permit the *gag* leader codons to be spliced in-frame to a long, *myb*-specific open reading frame beginning 115 codons upstream of the first internal *myb*-specific AUG (13).

The same antisera initially used to identify $p48^{v\text{-}myb}$ were also used to identify the chicken c-*myb* gene product. Three anti-*myb*-peptide antisera reacted with a M_r 110,000 cytoplasmic protein in normal chicken thymus (18), whereas the antiserum to the carboxyl-terminal, v-myb^{AMV}-encoded portion of a bacterial fusion protein recognized a M_r 75,000 nuclear protein in a transformed chicken erythroid cell line (19). Further, none of these antisera could identify *myb*-related proteins in other species.

The amino acid sequence predicted to be present in $p48^{v\text{-}myb}$ and which is encoded upstream of the first internal *myb*-specific AUG codon (Fig. 1) is also part of the c-*myb* region that is most conserved among metazoan species (6, 9–11, 20). We have therefore raised antibodies against this amino-terminal v-*myb* region, after its expression as part of a bacterial fusion protein, with the following objectives: (*i*) to ascertain that it is present in $p48^{v\text{-}myb}$ of AMV and in the transforming protein $p135^{gag\text{-}myb\text{-}ets}$ of E26 virus; (*ii*) to determine whether the molecular weight of the chicken c-*myb* protein is 75,000 or 110,000; and (*iii*) to identify the products of the c-*myb* gene in different metazoan species and determine their intracellular locations.

MATERIALS AND METHODS

Expression of a Conserved v-*myb* Domain in *Escherichia coli*. The conserved 5′ region of the v-*myb* oncogene was subcloned (Fig. 2) into an inducible, high-level *trpE* expression vector, pATH-3, supplied to us by T. J. Koerner and A. Tzagaloff (Columbia University, New York) using standard procedures (21). A sample of plasmid pUCMYB (to be described elsewhere) was digested to completion with *Nco* I [nucleotide (nt) 233] and *Xba* I (nt 1311), and the resulting v-*myb* fragment was purified (nucleotide numbering is according to ref. 7). Another sample of pUCMYB DNA was digested with *Kpn* I (nt 3) and *Eco*RI (nt 506), to obtain a fragment that contains the 5′ end of v-*myb* missing in the *Nco* I–*Xba* I fragment. Purified *Kpn* I–*Eco*RI fragment was digested with *Hin*fI, treated with Klenow fragment of DNA polymerase, and then digested with *Nco* I. This yielded a small fragment blunt-ended at the *Hin*fI (splice acceptor) site (nt 164) and extending to the *Nco* I site (nt 233). This fragment was purified by gel electrophoresis and added to a ligation mixture containing the *Nco* I–*Xba* I fragment of v-*myb* and pUC18 DNA digested with *Sma* I and *Xba* I. Minilysates of transformed *E. coli* HB101 were screened by restriction enzyme mapping to select plasmid pUC165X. Finally, plasmid pA340.A was constructed by inserting the 342-bp *Eco*RI

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Abbreviations: AMV, avian myeloblastosis virus; bp, base pair(s); kb, kilobase(s).

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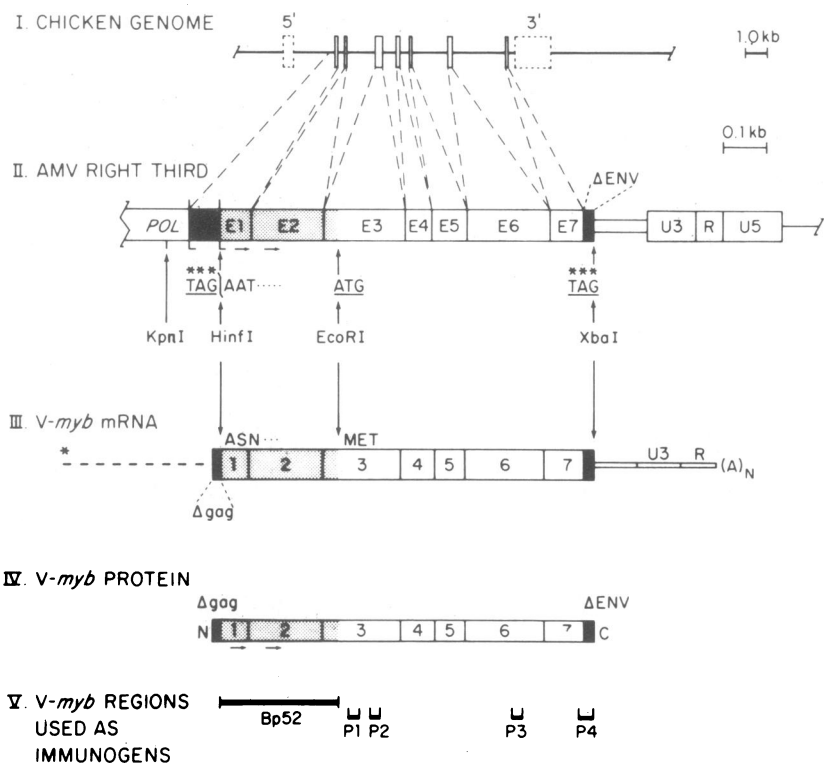

FIG. 1. Origin and structure of v-*myb*^{AMV}. (*I*) Chicken c-*myb* protooncogene. Boxes with solid outline represent exons transduced in AMV (6); boxes with dotted outline, c-*myb* coding information not present in AMV (12); horizontal lines, introns; kb, kilobase. (*II*) Transduced c-*myb* sequences. Part of an intron (dark box between *pol* and E1) and seven exons (E1–E7) have replaced most of the viral envelope gene (*env*) and the 3′ end of the viral polymerase gene (*pol*). Open reading frame [1146 base pairs (bp)] begins at AAT next to splice-acceptor site (13) and terminates at TAG provided by *env*. U3-R-U5 is the proviral long terminal repeat (LTR) composed of sequence unique to 3′ viral RNA terminus, short sequence repeated at both viral RNA termini, and sequence unique to 5′ viral RNA terminus. (*III*) v-*myb*^{AMV} subgenomic message starting within 5′ LTR (asterisk) includes the first 6 codons of the viral *gag* gene with initiation codon (Δ*gag*) (dark box) spliced to the transduced c-*myb* exons and the last 11 *env* codons plus TAG (Δ*env*) (dark box). (*IV*) Proposed structure of the 388 amino acid v-*myb* oncogene product, p48$^{v\text{-}myb}$. (*V*) Amino acid sequences used to generate anti-v-myb antisera: P1–P4, short peptides (14); Bp52, 115 amino acid sequence (shaded area) of E1, E2, and E3 located 5′ of the first v-*myb*-specific ATG that was inserted in plasmid pA340.A. Arrows represent a tandemly repeated sequence of 52 amino acids (15).

fragment of pUC165X into the single *Eco*RI site of plasmid pATH-3. The *trpE-myb* gene product of plasmid pA340.A, Bp52$^{trpE\text{-}myb}$ (Bp52), was expressed in *E. coli* C600 and purified by gel electrophoresis as described (16, 22).

Preparation of Anti-Bacterial Fusion Protein Antisera. New Zealand White rabbits were immunized by subcutaneous injections of emulsified gel slices containing 250 μg of Bp52 protein every 2 weeks as described (16). Anti-Bp52 antibodies were affinity-purified with 500 μg of Bp52, purified by gel electrophoresis, covalently linked to Affi-Gel 10 according to the suggestions of the manufacturer (Bio-Rad). After elution with 0.1 M glycine·HCl (pH 2.5), neutralization with Tris base, and addition of bovine serum albumin to a final concentration of 5 mg/ml, the anti-Bp52 immunoglobulin (IgG) was dialyzed against phosphate-buffered saline (0.15 M sodium chloride/15 mM sodium phosphate, pH 7.4) containing 0.01% sodium azide and then was stored at −70°C.

Rabbit anti-*myb* peptide 4 (P4) IgG was similarly affinity-purified, using Affi-gel 10 and 70 μg of gel-purified Bp43 protein (supplied by N. Kan and T. S. Papas), which is encoded by the carboxyl-terminal two-thirds of the v-*myb* open reading frame fused to a bacterial expression vector (23).

Cells. The AMV-transformed chicken myeloblastic line BM2 was provided by M. G. Moscovici and C. Moscovici (24). Other chicken cells were isolated and cultured as described (14, 25). Thymocytes were isolated from the thymus of 3-week-old BALB/c mice. The mouse myelomonocytic leukemia cell line WEHI-3B was provided by J. Ihle (National Cancer Institute) and cultured as described (26). Human hematopoietic cell lines from the erythroid (K-562), myeloid (KG-1 and HL-60), and lymphoid (CEM, IM-9, and HUT 79) lineages were supplied by O. Witte and I. Chen (University of California, Los Angeles) and cultured as described (27). The *Drosophila* embryonic cell line Kc was supplied by J. D. O'Connor (University of California, Los Angeles) and cultured as described (28).

Cell Labeling and Cell Fractionation. All cells were biosynthetically labeled with [^{35}S]methionine as described (14). *Drosophila* Kc cells were radioactively labeled in Seecoff's salt solution (pH 6.8) at 21°C. Labeled cells were fractionated into nuclei and cytoplasm, and the isolated nuclei were further fractionated into subnuclear compartments (18, 29).

Analysis of *myb*-Encoded Protein and mRNA. Protein encoded by either v-*myb* or c-*myb* was immunoprecipitated from detergent-solubilized lysates of labeled cells and analyzed by NaDodSO$_4$/PAGE as described (14).

Poly(A)$^+$ RNA was isolated, fractionated by electrophoresis in formaldehyde/agarose gels, and transferred to nitrocellulose (21). *myb*-related transcripts were detected by hybridization with the ^{32}P-labeled *Bam*HI insert of plasmid pHAX-4 (30) as described (13).

RESULTS

Presence of the Evolutionarily Conserved 5′ v-*myb* Domain in the Oncogene Products of AMV (p48$^{v\text{-}myb}$) and E26 Virus (p135$^{gag\text{-}myb\text{-}ets}$). The highly conserved, cell-derived 5′-terminal v-*myb*^{AMV} segment (Fig. 1) extending from the splice-acceptor site (*Hin*fI site) (13) to an *Eco*RI site located 9 bp 5′ of the first internal ATG codon of *myb* (6, 7) was subcloned into an inducible, high-level *trpE* expression vector plasmid (Fig. 2). This recombinant plasmid, pA340.A, should encode a protein containing a M_r 37,000 *trpE*-specific amino terminus fused to a M_r 15,000 *myb*-specific carboxyl terminus. Upon induction of the *trpE* operon in pA340.A-transfected bacteria, the predicted M_r 52,000 TrpE-myb fusion protein, Bp52, was produced abundantly (data not shown). Antisera from rabbits immunized with purified Bp52 protein reacted with both *trpE*- and *myb*-specific proteins, whereas the preimmunization sera did not (data not shown). Antibodies that were affinity-purified, by adsorption to and elution from a column of immobilized Bp52, specifically and solely immunoprecipitated p48$^{v\text{-}myb}$ from AMV-transformed myeloblasts, as did immunoaffinity-purified antiserum to v-*myb* carboxyl-terminal peptide (anti-P4) (Fig. 3*B*). The anti-Bp52-specific antibodies immunoprecipitated p48$^{v\text{-}myb}$ synthesized either by a cloned AMV-transformed myeloblast line (BM2) that contains only the defective AMV provirus (Fig. 3*B*, lane 2) or by polyclonal AMV-transformed peripheral blood leukemic myeloblasts (Fig. 3*B*, lane 4) that contain both the

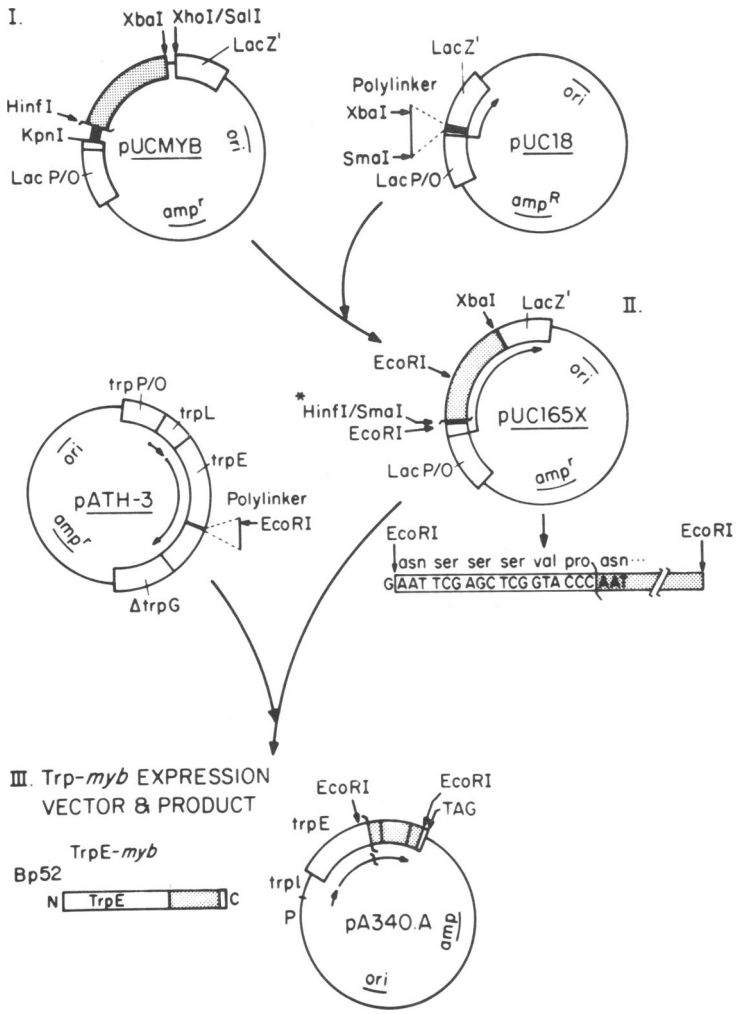

FIG. 2. Construction of the v-*myb* expression vector pA340.A. (*I*) Plasmid pUC165X was generated from pUCMYB by ligating the v-*myb* reading frame (stippled) into the polylinker of pUC18. (*II*) Plasmid pUC165X contains the entire v-*myb* reading frame joined in-frame (*) to the 11 amino-terminal codons of the pUC18 β-galactosidase gene. Utilizing *Eco*RI restriction sites located 18 bp 5′ of the *Hin*fI/*Sma* I junction and 9 bp 5′ of the internal v-*myb* ATG, a 342-bp fragment containing a highly conserved v-*myb* domain was isolated (shown below the plasmid). This fragment was inserted into the open reading frame of the *E. coli trpE* gene of plasmid pATH-3. (*III*) The resulting plasmid, pA340.A, encodes a M_r 52,000 TrpE-myb fusion protein.

AMV and helper proviruses. Both types of cells expressed the 7.2-kb AMV genomic RNA and the 2.0-kb subgenomic v-*myb* mRNA (Fig. 3*A*) (31, 32). The immunoprecipitation was completely blocked by preincubation of the antiserum with excess Bp52. These results show that $p48^{v\text{-}myb}$ contains the amino acid sequence encoded by the conserved region of $v\text{-}myb^{AMV}$, which is 5′ of the first *myb*-specific ATG. A similar analysis of E26 virus-transformed myeloid cells confirmed that the $p135^{gag\text{-}myb\text{-}ets}$ product of the 5.7-kb E26 virus genomic RNA (Fig. 3*A*, lane 3) (33) also contains a related amino acid sequence (Fig. 3*B*, lane 8), as predicted from DNA sequence analysis (5).

Presence of the Conserved 5′ v-*myb* Domain in the c-*myb* Product ($p75^{c\text{-}myb}$) of Normal Chicken Thymus. Previous studies had identified two candidate products of the chicken c-*myb* gene, a M_r 75,000 nuclear protein (16, 19) and a M_r 110,000 cytoplasmic protein (14, 18). To clarify this issue, we have used our new antiserum to identify the c-*myb* product in chicken thymocytes. Blot analysis revealed a *myb*-specific mRNA of 4.0 kb in normal thymus (Fig. 3*A*, lane 2), similar in size to that detected in hematopoietic cell lines transformed by either avian erythroblastosis virus (AEV) or Marek disease virus (8, 34). Immunoprecipitation of lysates of [^{35}S]methionine-labeled chicken thymocytes with anti-Bp52 antibodies specifically detected a M_r 75,000 protein (Fig. 3*B*, lane 6; Fig. 4*B*) as was found by Klempnauer *et al.* (16) in an AEV-transformed cell line. A M_r 75,000 protein was also immunoprecipitated from thymocytes by antisera (two) raised against the bacterially expressed carboxyl-terminal two-thirds of the v-*myb* protein (data not shown; antisera kindly provided by J. M. Bishop and T. S. Papas). The M_r 110,000 protein that we had previously observed with antisera against short *myb* peptides was not detected by our anti-Bp52 antibodies. Neither was any other crossreactive protein detected. Further, $p75^{c\text{-}myb}$ present in chicken thymocytes was localized within the nucleus (data not shown), as is $p75^{c\text{-}myb}$ in AEV-transformed erythroblasts (19), $p48^{v\text{-}myb}$, or $p135^{gag\text{-}myb\text{-}ets}$ (18, 19). Thus, the conserved 5′ domain of v-*myb* is also present in $p75^{c\text{-}myb}$. Neither 4.0-kb c-*myb* mRNA nor $p75^{c\text{-}myb}$ protein was observed in AMV- or E26 virus-transformed hematopoietic cells (Fig. 3).

Identification of c-*myb* Gene Products in Widely Divergent Metazoan Species. Precedents exist for the recognition of evolutionarily conserved protooncogene products by antibodies directed against highly conserved protein domains encoded by viral oncogenes. For instance, anti-$p21^{v\text{-}Ha\text{-}ras}$ immunoprecipitates a related protein in yeast (35), and anti-$pp60^{v\text{-}src}$ immunoprecipitates a homologous protein in *Drosophila* (36). The *myb* protein-coding domain present in $Bp52^{trpE\text{-}myb}$ is highly conserved in the DNA of a wide range of species, including *Drosophila*, mice, and humans in addition to chickens (6, 11, 20). We therefore examined appropriate cell lines and tissues from these metazoan species for the presence of candidate c-*myb* products.

In lysates prepared from adult *Drosophila* flies, which express a c-*myb*-related transcript of 3.8 kb (11), a protein of M_r 80,000 was detected by immunoblot analysis with our anti-Bp52 antibodies (data not shown). A M_r 80,000 protein was also specifically immunoprecipitated (Fig. 4*A*) from whole-cell extracts of the *Drosophila* embryonic cell line Kc (28).

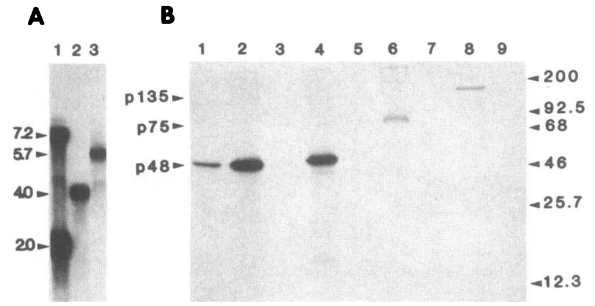

FIG. 3. Expression of *myb* in avian cells and tissues. (*A*) Blot hybridization of electrophoretically fractionated poly(A)$^+$ RNA isolated from cultured BM2 cells (AMV-transformed myeloblasts) (lane 1, 2 μg of RNA), 18-day chicken embryo thymus (lane 2, 10 μg), and E26-transformed peripheral blood chicken leukemic myeloblasts (lane 3, 6 μg) with ^{32}P-labeled v-*myb*-specific insert DNA isolated from plasmid pHAX-4 (31). Sizes of hybridizing RNAs are given at left, in kb. (*B*) Immunoprecipitation of the avian *myb* gene products in cells or tissues expressing *myb*-related mRNAs. Lysates prepared from metabolically labeled BM2 myeloblasts (lanes 1–3), AMV-transformed peripheral blood leukemic myeloblasts (lanes 4 and 5), chicken thymocytes (lanes 6 and 7), or E26-transformed peripheral blood leukemic myeloblasts (lanes 8 and 9) were immunoprecipitated with affinity-purified anti-v-*myb* carboxyl-terminal peptide (P4) IgG (lane 1), affinity-purified anti-Bp52 IgG (lanes 2, 4, 6, and 8), or affinity-purified anti-Bp52 IgG blocked with excess Bp52 (lanes 3, 5, 7, and 9). Positions of molecular weight markers ($M_r \times 10^{-3}$) run in parallel are at right.

In one transformed murine myeloid cell line (WEHI-3B) and in thymocytes from a 3-week-old mouse, which express a c-*myb* mRNA of 4.0 kb (36, 37) a labeled protein of M_r 75,000 was specifically immunoprecipitated by anti-Bp52 antibodies (Fig. 4 *C* and *D*). Immunoprecipitation of lysates of a human lymphoblastic cell line (CEM) revealed two molecular species of M_r 79,000 and 97,000, respectively (Fig. 4*E*). However, the M_r 97,000 protein was also detected in mature B-lymphoid (IM-9) cells, which do not express *myb*-related mRNA (see below), and it was not associated with the nuclear matrix/lamina fraction, as is p79$^{c\text{-}myb}$ (Fig. 5*A*).

Nuclear Localization of *myb*-Related Protein in Human and *Drosophila* Cells. The nuclear location of the candidate human p79$^{c\text{-}myb}$ and *Drosophila* p80$^{c\text{-}myb}$ further supports their relationship to the known products of the avian v-*myb* and c-*myb* genes. Subcellular fractionation and immunoprecipitation of human CEM cells, which express a high level of p79$^{c\text{-}myb}$, revealed that this nuclear protein has the same distribution among the various subnuclear compartments (Fig. 5*A*) as does p48$^{v\text{-}myb}$ in chicken leukemic cells (29). Under our fractionation conditions, approximately two-thirds of human

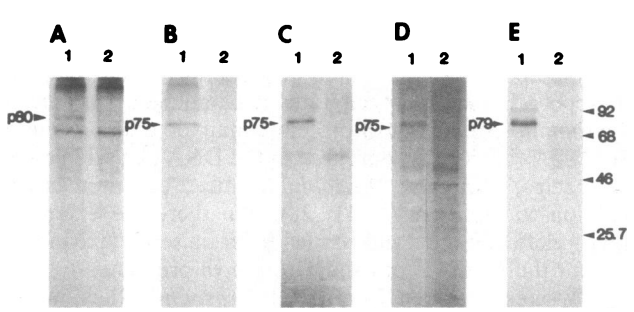

FIG. 4. Expression of c-*myb*-encoded proteins in *Drosophila* embryonic cell line Kc (*A*), chicken thymocytes (*B*), mouse thymocytes (*C*), mouse myeloid cell line WEHI-3B (*D*), and human lymphoblastic cell line CEM (*E*). Lysates of metabolically labeled cells were immunoprecipitated with affinity-purified anti-Bp52 IgG (lane 1) or with the same IgG blocked with excess Bp52 (lane 2).

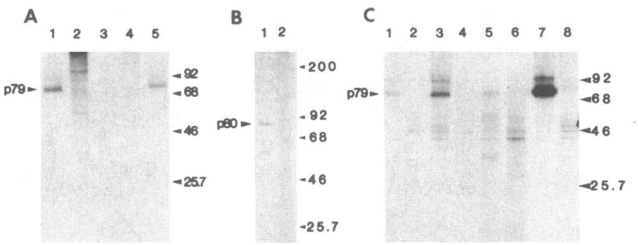

FIG. 5. Localization of the human and *Drosophila* c-*myb* gene product. (*A*) Immunoprecipitation of p79$^{c\text{-}myb}$ from nuclear and subnuclear lysates of CEM cells, using anti-Bp52 IgG. Lanes: 1, whole nuclei; 2 and 3, successive nucleoplasmic extracts; 4, chromatin fraction; 5, lamina/nuclear matrix fraction. (*B*) Specific immunoprecipitation of p80$^{c\text{-}myb}$ from the lamina/nuclear matrix fraction of Kc cells. Lanes: 1, anti-Bp52 IgG; 2, anti-Bp52 IgG blocked with Bp52. (*C*) Immunoprecipitation of p79$^{c\text{-}myb}$ from lysates of K-562 (lanes 1 and 2), KG-1 (lanes 3 and 4), HL-60 (lanes 5 and 6), and CEM cells (lanes 7 and 8), using either anti-Bp52 IgG (lanes 1, 3, 5, and 7) or anti-Bp52 IgG blocked with Bp52 (lanes 2, 4, 6, and 8).

p79$^{c\text{-}myb}$ is tightly associated with the insoluble peripheral lamina/nuclear matrix. The remaining third is present in the soluble nucleoplasmic fraction.

Subnuclear fractionation of labeled Kc cells revealed that the putative M_r 80,000 *Drosophila* c-*myb* product has the same subnuclear compartmentalization as the products of the chicken and human *myb* genes and was not detected in the cytoplasm (data not shown). Further, this protein is the only molecular species immunoprecipitated by our anti-Bp52 antiserum from lysates of solubilized nuclear matrix fractions isolated from labeled Kc nuclei (Fig. 5*B*).

The Product of the Human c-*myb* Gene is Expressed in Immature Erythroid, Myeloid, and Lymphoid Hematopoietic Lineages. The presence or absence of c-*myb* mRNA in numerous human hematopoietic cell lines has been determined (27), and we have analyzed a number of these cell lines for the expression of a c-*myb* product that contains the conserved *myb* domain present in Bp52. A M_r 79,000 protein was specifically immunoprecipitated from K-562 (erythroblasts), KG-1 (myeloblasts), and HL-60 (promyelocytes) cells (Fig. 5*C*) as well as from CEM (lymphoblasts) cells, which express c-*myb* mRNA (27), but not from IM-9 (mature B-lymphocytic) or HUT 79 (mature T-lymphocytic) cells (data not shown), which do not express *myb*-related mRNA (27). This pattern of p79$^{c\text{-}myb}$ expression correlates precisely with *myb*-specific mRNA (27) and demonstrates that this protein can be expressed in the three major hematopoietic cell lineages. As mentioned above, the fainter M_r 97,000 band detected in immunoprecipitates of all human leukemic cells tested does not appear to be *myb*-specific.

DISCUSSION

Cellular nucleic acid sequences closely related to the *myb* oncogenes of AMV and E26 leukemia virus have been conserved among metazoan species (9, 10, 11, 20). Herein we have demonstrated that this evolutionary conservation is maintained at the protein level. Antibodies raised against the *myb* domain encoded by the 5′ one-third of the v-*myb* long open reading frame, upstream of the first internal ATG codon of v-*myb*^{AMV}, immunoprecipitated p48$^{v\text{-}myb}$ with high affinity and specificity. This demonstrates that this protein domain is present in p48$^{v\text{-}myb}$, as previously hypothesized from the unexpected larger size of the protein and from nuclease S1 mapping of the v-*myb* splice-acceptor site (13).

Comparison of this 115 amino acid v-*myb* sequence derived from chicken DNA with the amino acid sequence deduced from the nucleotide sequence of the c-*myb* genes of various

examined species indicates that antibodies to this antigen should crossreact with the c-*myb* products of other species. This 5'-terminal v-myb^{AMV} protein coding domain is 96% identical (at the amino acid level) with the corresponding mouse c-*myb* domain (20), 97% identical with chicken c-*myb* and v-myb^{E26} (5, 6), and 71% identical with *Drosophila* c-*myb* (11). Even between the more distantly related sequences present in the chicken and the fruit fly, several uninterrupted regions of 10–20 identical amino acids are present. The sequence of human c-*myb* has yet to be reported, but the amino acid sequence of the conserved domain described above is likely to be nearly identical to that of the mouse and chicken.

We have confirmed that the product of the chicken c-*myb* gene is a M_r 75,000 nuclear protein, as reported by others (16, 19), and not $p110^X$, a hematopoietic-specific product of an unknown gene (14). In addition, we have identified a family of *myb*-related nuclear proteins of M_r 75,000–80,000 in human, mouse, and *Drosophila*. In humans, this *myb*-related protein is present in immature cells of the lymphoid, erythroid, and myeloid lineages.

Our conclusion that these proteins are the products of their respective c-*myb* genes is based on the following observations: (*i*) antisera from rabbits immunized with the Bp52 TrpE-myb fusion protein immunoprecipitate $p75–80^{c\text{-}myb}$, as well as $p48^{v\text{-}myb}$ and $p135^{gag\text{-}myb\text{-}ets}$, but do not do so after preincubation with excess antigen; (*ii*) immunoaffinity-purified antibodies solely and specifically recognize $p48^{v\text{-}myb}$, $p135^{gag\text{-}myb\text{-}ets}$, and chicken $p75^{c\text{-}myb}$; (*iii*) detection of $p75–80^{c\text{-}myb}$ with our antiserum correlates precisely with expression of c-*myb* mRNA in various cell types; and (*iv*) all of these *myb*-sequence-containing proteins are located in the cell nucleus, as are $p48^{v\text{-}myb}$ and $p135^{gag\text{-}myb\text{-}ets}$. Further, in human and *Drosophila* cells a major portion of the *myb*-related proteins is associated with an insoluble nuclear structure. This observed intranuclear localization, which is dependent upon the fractionation procedures used, is similar to that previously reported for the products of the v-*myb*, v-*myc*, and c-*myc* genes (12, 29, 37).

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