MOLECULAR AND CELLULAR BIOLOGY, Nov. 1985, p. 3017–3023
0270-7306/85/113017-07$02.00/0
Copyright © 1985, American Society for Microbiology
Vol. 5, No. 11

Nuclear Compartmentalization of the v-*myb* Oncogene Product

WILLIAM J. BOYLE,[1] MARTHA A. LAMPERT,[1] ANDREW C. LI,[2] AND MARCEL A. BALUDA[1]*

Jonsson Comprehensive Cancer Center and Department of Pathology,[1] and Department of Medicine,[2] UCLA School of Medicine, Los Angeles, California 90024

Received 30 May 1985/Accepted 23 August 1985

Nuclei obtained from chicken leukemic myeloblasts transformed by avian myeloblastosis virus were fractionated into various subnuclear compartments, which were then analyzed by specific immunoprecipitation for the presence of the leukemogenic product, p48$^{v\text{-}myb}$, of the viral oncogene. In cells labeled for 30 or 60 min with L-[^{35}S]methionine and in unlabeled exponentially dividing leukemic cells analyzed by Western blotting, p48$^{v\text{-}myb}$ was detected within the nucleoplasm (29 ± 9% [standard deviation] of the total), chromatin (7 ± 4%), and lamina-nuclear matrix (64 ± 9%). Also, in myeloblasts analyzed by immunofluorescence during mitosis, p48$^{v\text{-}myb}$ appeared to be dispersed through the cell like the lamina-nuclear matrix complex. Strong attachment to the nuclear matrix-lamina complex suggests that p48$^{v\text{-}myb}$ may be involved in DNA replication or transcription or both.

The acutely oncogenic, replication-defective avian myeloblastosis virus (AMV) induces acute myeloblastic leukemia exclusively in chickens and transforms only myeloid cells in vitro (2, 35). The leukemogenic properties of AMV are encoded by v-*myb*, which arose by recombination between the replication-competent myeloblastosis-associated virus type 1 and highly conserved proto-*myb* cellular genetic elements (36, 37, 44). Based on differences in gene, transcript, and protein structures (7, 25–27, 40), v-*myb* is an altered form of proto-*myb*, containing internal proto-*myb* sequences. The absence of a significant portion of the proto-*myb* structure in v-*myb* may account for its transforming potential.

The v-*myb* oncogene product, p48$^{v\text{-}myb}$, is a nuclear protein with an M_r of 48,000 (6, 28) and a half-life of 30 min (J. M. Ong and M. A. Baluda, unpublished data) that presumably mediates specific transformation of myeloid cells through interactions with nuclear components (2, 28). p48$^{v\text{-}myb}$ is not glycosylated, is phosphorylated to a small extent, and does not act as a kinase in immunoprecipitates (W. J. Boyle, J. M. Ong, and M. A. Baluda, unpublished data). Also, there is no apparent structural similarity between p48$^{v\text{-}myb}$ and the products of the three known classes of nonnuclear retroviral oncogenes; i.e., those derived from soluble external growth factors such as p28^{sis} (47), those representing altered plasma membrane growth factor receptors and tyrosyl protein kinases such as gp74$^{erb\text{-}B}$ and pp60^{src} (16, 22), and those related to plasma membrane-associated proteins that appear to modulate adenyl cyclase activity such as p21^{ras} (46). The AMV p48$^{v\text{-}myb}$ and the oncogene product (p135) of the other *myb*-containing retrovirus, E26 virus (6), as well as the products of the v-*myc* (1, 15) and v-*fos* (14) oncogenes, constitute a fourth class of nuclear transforming proteins (21). The functions of these nuclear transforming proteins are not known, but their subcellular localization suggests perturbations at the DNA level.

Previously we have shown that a nuclear fraction isolated from AMV-transformed myeloblasts contains virtually all of p48$^{v\text{-}myb}$ (6). We have now sought to identify the subnuclear association of p48$^{v\text{-}myb}$ by a fractionation procedure that yields relatively well-defined nuclear components after sequential extraction with nonionic detergents, nuclease, and high-salt buffer (20). Here we report the effects of this serial fractionation on nuclear ultrastructure and DNA, protein, and p48$^{v\text{-}myb}$ distribution. We detected p48$^{v\text{-}myb}$ in three subnuclear compartments, including a very tight association with the nuclear matrix-lamina complex, which is generally thought to be intimately associated with DNA replication and transcription (41, 43).

MATERIALS AND METHODS

Cells. The BM2 cell line of chicken myeloblasts transformed by AMV was the gift of Giovannella and Carlo Moscovici. Cells were cultured and biosynthetically labeled with L-[^{35}S]methionine (specific activity, 1,100 Ci/mmol; New England Nuclear Corp.) as previously described (6, 7).

Nuclei isolation and fractionation. Intact nuclei were isolated from BM2 myeloblasts as previously described (6) with minor modifications. Cells were lysed with ice-cold nuclear buffer containing 5 mM sodium phosphate (pH 7.4), 50 mM sodium chloride, 150 mM sucrose, 5 mM potassium chloride, 2 mM dithiothreitol, 1 mM magnesium chloride, 0.5 mM calcium chloride, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), and 0.1% Kyro-EOB (Proctor and Gamble). Nuclei were then collected from lysed cells on a cushion of 30% sucrose containing TN buffer (2.5 mM Tris hydrochloride, pH 7.4, 10 mM sodium chloride) (9) after centrifugation at 1,000 × *g* for 10 min at 4°C. The sequential extraction of intact nuclei was a modified procedure described by Razin et al. (39) and Bodnar et al. (5). After centrifugation, 5×10^6 to 10×10^6 Kyro-EOB-isolated nuclei were suspended in 1.0 ml of ice-cold Nonidet P-40 (NP40) buffer (0.5% NP40, 10 mM sodium phosphate, pH 7.4, 120 mM NaCl, 0.1 mM PMSF) and kept for 30 min on ice. After NP40 treatment the nuclei were collected by gentle pelleting at 500 × *g* and then reextracted twice by the same procedure. In different experiments all three supernatants representing the nucleoplasmic fraction were either pooled or kept separate. The final NP40 nuclear pellet was suspended in 100 μl of nuclease digestion buffer (20 mM Tris hydrochloride, pH 7.4, 100 mM NaCl, 50 mM KCl, 5 mM $CaCl_2$, 0.1 mM EDTA, 0.1 mM PMSF) containing 170 U of micrococcal nuclease (Boehringer-Mannheim) per ml and incubated for 30 min at 37°C. Nuclease digestion was stopped by the addition of 900 μl of high-salt buffer (2.0 M NaCl, 0.5% NP40, 5 mM sodium phosphate (pH 7.4), 10 mM EDTA). Soluble chromatin was

* Corresponding author.

extracted by incubation for 60 min on ice and separated from the residual nuclear matrix by centrifugation over a cushion of 30% sucrose containing TN buffer at 12,000 × *g* for 15 min. The soluble supernatant (chromatin fraction) was removed, and the insoluble pellet was redigested and reextracted with high-salt buffer and collected by centrifugation as before. The resulting insoluble nuclear matrix-lamina fraction was solubilized by boiling in 100 μl of electrophoresis buffer (29) and then diluted with 10 volumes of detergent lysis buffer (7). In some experiments, low-salt buffer (50 mM NaCl, 0.5% NP40, 5 mM sodium phosphate, pH 7.4, 10 mM EDTA) was used instead of high-salt buffer after nuclease treatment.

To examine total nuclear proteins in fractions extracted from unlabeled nuclei, fractions were precipitated with 20% trichloroacetic acid (TCA). The pellets were washed in absolute acetone, solubilized in sample buffer, and then electrophoresed on 7.5 to 15% linear polyacrylamide-acrylamide gradient slab gels. For quantitation of DNA in subnuclear fractions, BM2 cells were labeled with [^{3}H]thymidine for at least 12 h before nuclear isolation and fractionation. Fractions were made 10% in TCA, and TCA-insoluble tritiated DNA was quantitated by liquid scintillation counting.

Electron microscopy. Whole cells, isolated intact nuclei, NP40-extracted nuclei, and residual nuclear matrix-lamina were prepared for electron microscopy by standard procedures (34). Briefly, samples were incubated in 0.1 M sodium cacodylate buffer (pH 7.4) containing 0.1 M sucrose and 2% (vol/vol) glutaraldehyde (Polysciences, Inc.) for 1 h at 4°C. Fixed material was pelleted by centrifugation, washed in cacodylate-sucrose buffer, and incubated for 1 h at 4°C in postfixation buffer (0.1 M sodium cacodylate, 0.1 M sucrose, 1% [wt/vol] osmium tetroxide). Samples were dehydrated in increasing concentrations of ethanol before being embedded in a mixture of Epon and Araldite (Polysciences Inc.). Blocks were sectioned with an ultratome (LKB type 4802A), and "silver" thin sections were collected on Formvar-coated copper grids and stained with uranyl acetate and lead citrate. Samples were viewed and photographed with a Philips 300 electron microscope at 60 kV.

Immunoprecipitation and immunofluorescence. The anti-*myb* peptide P4 antiserum is a polyclonal rabbit antiserum prepared against the 19 carboxy-terminal amino acids of the v-*myb* oncogene (40). Preparation of this antipeptide antiserum and its immune activity have been described (7, 37). Human antilamin antiserum LS-1 (31) was kindly provided by Frank McKeon and Marc Kirshner (University of California, San Francisco).

Immunoprecipitation from radiolabeled cell lysates and electrophoretic analysis of immunoprecipitates were done as previously described (7) with minor modifications. Nuclear lysates (1 ml) containing extract from 10^7 nuclei were incubated with 5 μl of anti-*myb* antiserum and precipitated with 20 μl of protein A-coated Sepharose beads (Pharmacia Fine Chemicals). The radioimmunoprecipitated protein was quantitated by densitometric scanning of autoradiograph bands (Beckman DU-7 spectrophotometer). Immunofluorescent analysis of BM2 cells was performed as previously described (6). Immunoblot analysis of nuclear extracts was done essentially as described previously, with radioiodinated protein A used to detect immune complexes (11, 24).

RESULTS

Chemical fractionation of intact nuclei isolated from AMV-transformed myeloblasts. Nuclei isolated from BM2 cells with Kyro-EOB were sequentially fractionated by the successive chemical treatments described in Materials and Methods. The resulting structural alterations of the nuclei are shown in Fig. 1. Electron micrographs of untreated BM2 cells and of nuclei isolated from Kyro-EOB-lysed cells revealed similar nuclear structure, indicating that the isolated nuclei remained intact (Fig. 1A and B). In particular, they had an apparently undamaged nuclear envelope with discontinuous regions that resembled pore complexes, a contiguous peripheral lamina, and undegraded nucleoli characteristic of those seen in whole cells. Also, such isolated nuclei were almost free of cytoplasmic vesicles. Since we relied on the gentle, nonionic Kyro-EOB detergent to lyse the plasma membrane (6), it was not necessary to apply hypotonic or hypertonic buffers, which might have distorted the nuclei during osmotic shifts. Analysis by polyacrylamide gel electrophoresis (PAGE) of the total nuclear protein content revealed a pattern typical for nuclei, with prominent bands corresponding to histones, intermediate filaments, lamins, actin, and myosin (Fig. 2, lane 1).

Extraction of isolated nuclei with 0.5% NP40 buffer dissolved the nuclear envelope membranes but did not appear to disturb the peripheral lamina (Fig. 1C). The overall nuclear architecture of NP40-treated nuclei was preserved, although there was a conspicuous loss of homogeneous nuclear sap, resulting in electron-translucent cavities. The protein-rich supernatant fraction obtained by NP40 treatment, which we call the nucleoplasm, contained less than 1% of the total nuclear DNA (not shown) and was virtually free of chromatin-associated histone proteins (Fig. 2, lane 2).

Digestion of these NP40-treated nuclei with micrococcal nuclease, followed by extraction with high-salt buffer, released approximately 90% of the total DNA (not shown) along with all of the chromatin-associated histone proteins (Fig. 2, lane 3). After this series of rather harsh treatments, there remained an insoluble subnuclear structure (Fig. 1D) which contained less than 10% of the total DNA (not shown). Analysis of the proteins in this fraction showed a pattern of high-molecular-weight polypeptides (Fig. 2, lane 4; also see Fig. 4, lane 4) characteristic of the nuclear matrix-lamina complex of mammalian and avian cells, such as the p70 and p68 lamin components (8, 17). Depending on whether nuclease treatment preceded or followed the high-salt treatment, 90 or 98%, respectively, of the labeled DNA was removed from the NP40-extracted nuclei (not shown). Electron micrographs of the remaining nuclear components revealed a filamentous, basketlike structure characteristic of the nuclear matrix-lamina complex (12) (Fig. 1D). Thus, by the criteria of DNA depletion, histone depletion, presence of characteristic proteins, including lamins, and ultrastructure, this subnuclear fraction appears to be the peripheral lamina-nuclear matrix (4, 20).

Subnuclear localization of p48$^{v\text{-}myb}$. Antibodies that specifically recognize the carboxyl terminus of p48$^{v\text{-}myb}$ were used to probe the various subnuclear fractions described above. Three types of experiments were carried out with exponentially replicating leukemic cells having a division time of 14 h: (i) cells were labeled with L-[^{35}S]methionine for 30 min, which is equal to one half-life of p48$^{v\text{-}myb}$; (ii) cells were labeled in the same manner but for 60 min; and (iii) unlabeled cells were used for analysis by Western blotting. In each experiment the nuclei were fractionated, and in each fraction the amount of p48$^{v\text{-}myb}$ was determined as described in Materials and Methods. The amount of DNA in each subnuclear fraction was measured in parallel fractionations of cells labeled with [^{3}H]thymidine for 12 h. p48$^{v\text{-}myb}$ was

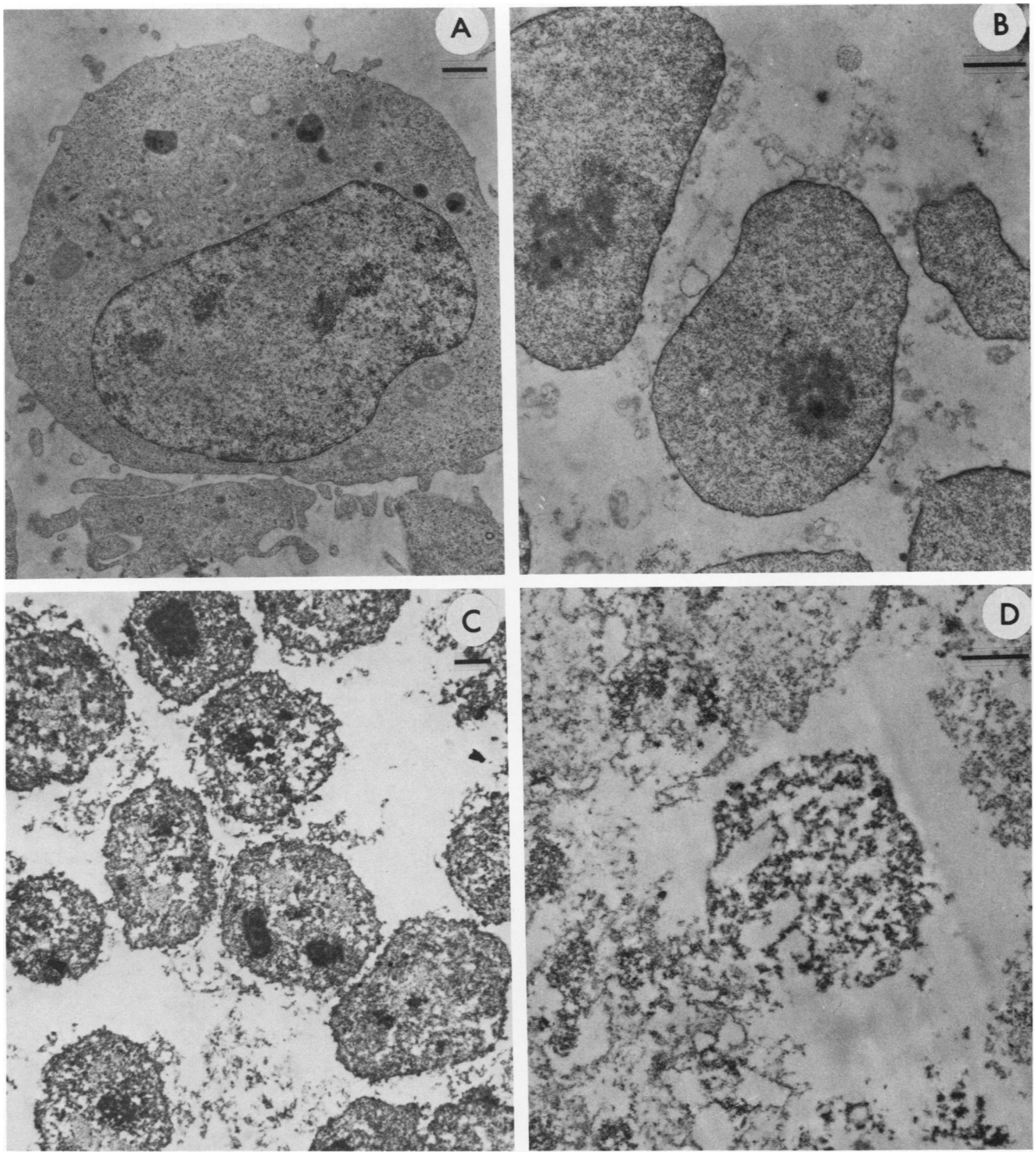

FIG. 1. Ultrastructure of nuclear fractions. Thin-section electron microscopy of BM2 myeloblasts treated sequentially as described in Materials and Methods. (A) Whole cells. (B) Isolated nuclei from Kyro-EOB-lysed cells. (C) Nuclei shown in panel B after NP40 extraction. (D) Nuclei shown in panel C after two cycles of digestion with micrococcal nuclease and incubation in high-salt buffer. Bars, 1 μm.

detected in three nuclear fractions (Table 1): of the total p48$^{v\text{-}myb}$, 29% was in the nucleoplasm, 7% was in the chromatin, and 64% was in the nuclear matrix-lamina complex.

In three separate experiments in which nuclei were isolated from cells labeled with L-[^{35}S]methionine for 30 min, 34 ± 9% (mean ± standard deviation) of the total p48$^{v\text{-}myb}$ was removed by two successive extractions in NP40 buffer

(nucleoplasmic fraction) (Fig. 3). In two experiments in which the cells were labeled with L-[^{35}S]methionine for 60 min and treated identically, 25 ± 8% of the total p48$^{v\text{-}myb}$ was removed. Finally, in one experiment in which unlabeled cells were fractionated, the proteins in each subnuclear fraction were separated by electrophoresis and transferred to nitrocellulose, and then p48$^{v\text{-}myb}$ was detected with specific antiserum and ^{125}I-labeled protein A, 25% of the total p48$^{v\text{-}myb}$ was detected in the nucleoplasmic fraction. Less than 1% of the total DNA was present in the nucleoplasmic fraction. The p48$^{v\text{-}myb}$ present in the nucleoplasm could represent newly synthesized molecules or unbound molecules in equilibrium between free and bound states. Alternatively, p48$^{v\text{-}myb}$ could have been trapped in the small population of vesicles adhering to the nuclear membrane. However, our immunofluorescent analysis of intact cells with the same antipeptide antibody did not support this hypothesis (6) (see Fig. 5A).

TABLE 1. Percentage of total p48$^{v\text{-}myb}$ present in subnuclear fractions of AMV-transformed myeloblasts[a]

Prepn tested (no. of expts)	% (mean ± SD) of total p48 $^{v\text{-}myb}$ in:		
	Nucleoplasm	Chromatin	Matrix-lamina
^{35}S labeled, 30 min (3)	34 ± 9	5 ± 2	62 ± 11
^{35}S labeled, 60 min (2)	25 ± 8	7 ± 3	68 ± 5
Steady state[b] (1)	25	11	64
All experiments (6)	29 ± 9	7 ± 4	64 ± 9

[a] In all experiments, exponentially dividing myeloblasts were treated under identical conditions. Radiolabeling was done with the same concentration of L-[^{35}S]methionine. After immunoprecipitation, PAGE, and autoradiography, the intensity of each p48 band was measured by densitometer scanning.

[b] For this experiment, unlabeled cells were fractionated under the same conditions as the radiolabeled cells, and p48 was detected in the subnuclear fractions by Western blotting and probing with antiserum and ^{125}I-protein A.

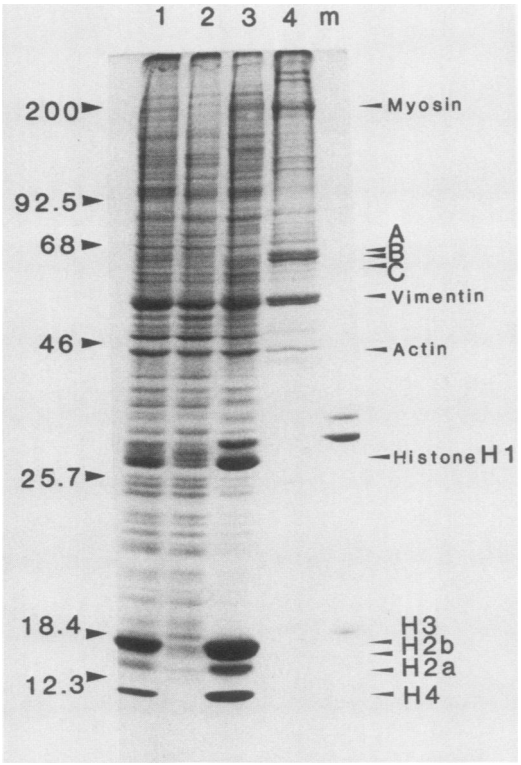

FIG. 2. Sodium dodecyl sulfate (SDS)-PAGE of nuclear proteins. Proteins in the various subnuclear fractions were separated on a linear 7.5 to 15% gradient gel and stained with Coomassie blue. Supernatants and solubilized pellets were precipitated with 20% TCA, and the precipitates were washed in absolute acetone before being dissolved in electrophoresis buffer. Lanes: 1, total nuclear lysate from whole nuclei (shown in Fig. 1B); 2, supernatant obtained after extraction of isolated nuclei with nuclear buffer containing 0.5% NP40; 3, supernatant released from NP40-extracted nuclei after nuclease and high-salt buffer treatment; 4, nuclease-resistant, high-salt-resistant nuclear skeleton solubilized by boiling in electrophoresis buffer and dilution in high-detergent lysis buffer (6); m, micrococcal nuclease (amount equivalent to that added during preparation of the sample shown in lane 3). Histone core proteins and H1, as well as the structural proteins actin, vimentin, and myosin, are indicated. A to C, Lamina structural components (lamins) p70, p68, and p62, respectively. Extract was obtained from 1×10^6 (lanes 1 and 2) or 5×10^6 (lanes 3 and 4) nuclei. Molecular weights (in thousands) are shown to the left.

After removal of the nucleoplasm with NP40, an additional 7 ± 4% of the total p48$^{v\text{-}myb}$ was released from the nuclei by nuclease digestion and extraction with high-salt buffer (Fig. 3, Table 1). In this so-called chromatin fraction, the histone proteins and 90 to 95% of the ^{3}H-labeled TCA-precipitable DNA were also released. The small percentage of p48$^{v\text{-}myb}$ in this fraction could represent p48$^{v\text{-}myb}$ specifically bound to chromatin or trapped in this fraction. The resulting chromatin-depleted, insoluble nuclear matrix-lamina complex contained 64 ± 9% of the total p48$^{v\text{-}myb}$ (Fig. 3, Table 1) and less than 10% of the total cellular DNA.

The reliability of our fractionation technique was tested further by using human antisera directed against the lamin A and C components (31), which cross-react with avian lamins (28), to demonstrate the presence of such antigens in our lamina-nuclear matrix fraction (Fig. 4, lane 4). Thus, nearly

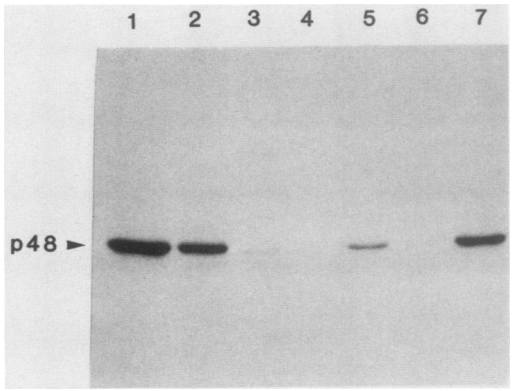

FIG. 3. Identification of p48$^{v\text{-}myb}$ in the various subnuclear fractions of leukemic cells labeled with L-[^{35}S]methionine for 30 min. Immunoprecipitates of subnuclear fractions were washed and eluted as previously described (6) and then analyzed by PAGE on a 10% SDS-polyacrylamide gel containing 0.5 M urea. For autoradiography the salicylated dried gel was exposed to film for 20 h. Lanes: 1, total nuclear lysate, 2, 3, and 4, supernatant extracted from isolated nuclei after one, two, and three consecutive incubations, respectively, in buffer containing 0.5% NP40; 5 and 6, chromatin-containing supernatant released from NP40-extracted nuclei after one or two treatments with micrococcal nuclease and high-salt buffer, respectively; 7, clarified supernatant of solubilized lamina-nuclear matrix pellet. Arrow, Migration site of p48$^{v\text{-}myb}$.

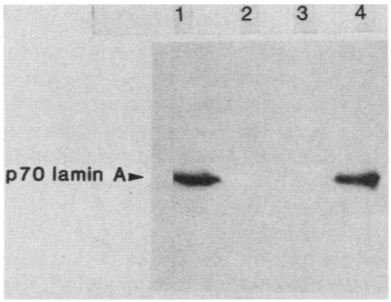

FIG. 4. Identification of nuclear lamin proteins in subnuclear fractions. Subnuclear fractions obtained from the equivalent of 5×10^6 nuclei were separated by SDS-PAGE on 10% gels and then electrophoretically transferred to nitrocellulose at 40 V for 16 h. The nuclear lamin proteins were detected with a 1:1,000 dilution of human antilamin antisera and ^{125}I-labeled protein A. Shown is an autoradiograph of a filter exposed to film for 5 h. Lanes: 1, total nuclear proteins; 2, pooled nucleoplasmic fractions obtained by three consecutive NP40 extractions of isolated nuclei; 3, pooled chromatin fractions obtained by two successive treatments of NP40-extracted nuclei with micrococcal nuclease and high-salt buffer; 4, peripheral lamina-nuclear matrix solubilized in high-detergent buffer. Arrow, Migration site of the 70,000-M_r lamin A (33).

two-thirds of the p48$^{v\text{-}myb}$ was tightly associated with material representing the nuclear framework.

Dispersion of p48$^{v\text{-}myb}$ in dividing leukemic cells. During immunofluorescent analysis of exponentially replicating leukemic myeloblasts with anti-*myb* P4 antibodies, we noted that in dividing cells, p48$^{v\text{-}myb}$ was dispersed throughout the cytoplasm in a granular pattern and appeared to be excluded from condensed metaphase chromosomes (Fig. 5). During interphase, p48$^{v\text{-}myb}$ was detected homogeneously throughout the nucleus (Fig. 5A), except in the nucleoli. Nuclear matrix components such as the lamins show a similar pattern during the cell cycle (19, 31, 32), dispersing during mitosis in a diffuse cytoplasmic pattern surrounding chromosomes. This pattern of immunofluorescent staining of p48$^{v\text{-}myb}$, along with the fractionation data, suggests that the AMV oncogene product is not associated with the bulk of chromatin.

DISCUSSION

Previously we reported that the v-*myb* oncogene product, p48$^{v\text{-}myb}$, is located within the nuclei isolated from either freshly explanted or cultured leukemic cells transformed by AMV (6, 30). In this study we fractionated such nuclei into the subnuclear compartments defined previously by their ultrastructure and macromolecular content (4, 8, 12, 20). The leukemogenic p48$^{v\text{-}myb}$ was present in three distinct subnuclear fractions, corresponding to nucleoplasm (29%), chromatin (7%), and lamina-nuclear matrix (64%). This distribution indicates that p48$^{v\text{-}myb}$ is involved in different types of nuclear associations and possibly in different functions.

The nucleoplasmic form of p48$^{v\text{-}myb}$, which represented almost one-third of the total p48$^{v\text{-}myb}$, was easily removed from nuclei under nondenaturing conditions and retained its DNA-binding property (33; data not shown). Since immunoaffinity-purified p48$^{v\text{-}myb}$ can bind directly to DNA (33), accessory proteins are apparently not required for DNA binding. Consonant with this property, we have previously reported the presence within the v-*myb* amino acid sequence of a potential DNA-binding region that resembles a consensus domain in bacterial, bacteriophage, and eucaryotic DNA-binding proteins (30). Because it is easy to extract, the nucleoplasmic form of p48$^{v\text{-}myb}$ may not yet have interacted with potential DNA- or matrix-binding substrates.

The low concentration of p48$^{v\text{-}myb}$ within the chromatin fraction (7%) is somewhat surprising, since it has DNA-binding potential. However, p48$^{v\text{-}myb}$ may interact directly with DNA only at specific sites of active replication or transcription. Our analysis of nuclear fractions indicates that most of the v-*myb* protein is associated with nuclear components (nucleoplasm and matrix) which contain only 10% of the total nuclear DNA. Furthermore, immunofluorescent staining of cells undergoing mitosis with anti-*myb* antisera did not detect p48$^{v\text{-}myb}$ in association with condensed chromatin. It remains to be determined whether the small amount

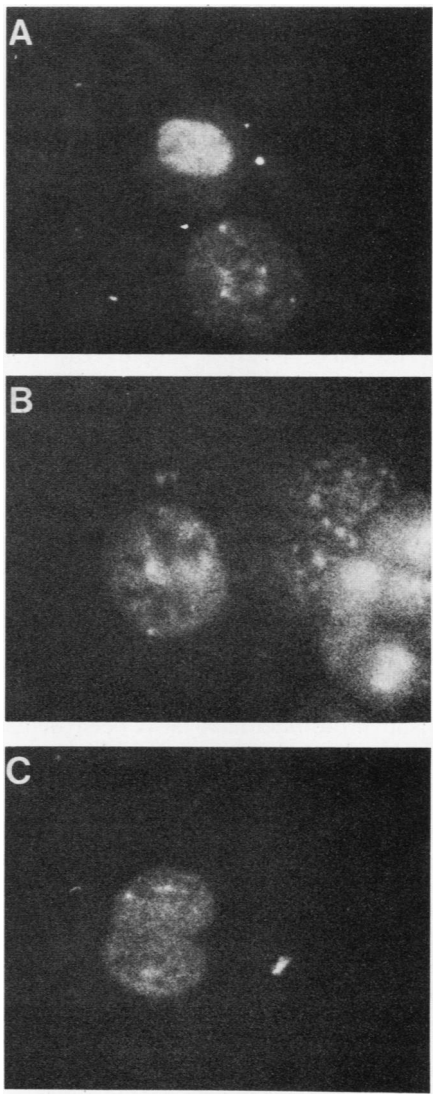

FIG. 5. Indirect immunofluorescent microscopy of dividing leukemic myeloblasts. Exponentially replicating BM2 cells were fixed on glass slides, permeabilized with acetone, and treated with rabbit anti-*myb* P4 antiserum and goat anti-rabbit immunoglobulin G conjugated to fluorescein. (A) Cell during interphase (top) and mitosis (bottom). (B) Cell during metaphase (center). The adjacent clump contains both dividing and nondividing cells. (C) Myeloblast in late telophase.

of p48$^{v\text{-}myb}$ within the chromatin fraction represents a specific population interacting directly with chromatin or contamination from the other nonchromatin nuclear fractions.

Our localization of nearly two-thirds of the total p48$^{v\text{-}myb}$ in the nuclear matrix-lamina fraction is supported by the colocalization of lamina structural proteins and by the granular and diffuse cytoplasmic dispersion of p48$^{v\text{-}myb}$ in dividing cells (19, 31). It appears unlikely that the association of p48$^{v\text{-}myb}$ with this fraction is an artifact due to precipitation of the protein caused by DNase digestion and high-salt buffer treatment. The use of low (0.05 M) salt concentrations after DNase treatment or of high salt concentrations before DNase treatment did not affect the fraction of p48 retained by the lamina-nuclear matrix. In eucaryotic cells, the nuclear matrix and peripheral lamina are structural entities where both replication and transcription of DNA occur (3, 13). Several nonlamin matrix protein components have been identified that bind DNA at chromatin attachment sites within the lamina-matrix and protect it from nucleolytic enzymes (5). Nuclease-resistant chromatin loop structures bound to the matrix harbor genes that are being actively transcribed (13). Identification of a lamina-matrix bound population of p48$^{v\text{-}myb}$ raises the possibility that p48$^{v\text{-}myb}$ is involved in these essential cellular functions. The short half-life of p48$^{v\text{-}myb}$ is consonant with its being a regulatory protein.

Localization of a transforming viral gene product within the lamina-nuclear matrix is not unique to AMV. The E1a product of adenovirus (18), the large T antigen of simian virus 40 and polyoma virus (8, 45), p110$^{gag\text{-}myc}$ of MC-29 virus (17), and ICP8 of herpes simplex virus (38) are also found in this subnuclear compartment. The E1a protein has been implicated as a *trans*-acting transcriptional activator (18), and the large T antigen and the ICP8 protein are DNA-binding proteins involved in the replication of viral DNA genomes (12, 38). ICP8 has been shown to shuttle between the peripheral lamina-nuclear matrix and replicating, chromatin-associated viral DNA (38), as does alpha-DNA polymerase with cellular DNA in rat liver hepatocytes located at the proliferative margin of regenerating liver (42). Thus, both transcriptional and replicative DNA events mediated by these viral and cellular proteins appear to occur at sites where chromatin loop structures are anchored to the matrix. Like p48$^{v\text{-}myb}$, the nuclear phosphoproteins encoded by v-*myc* and proto-*myc* have also been detected in the nuclear matrix complex (17) and have been shown to bind DNA directly (48).

To understand the role of p48$^{v\text{-}myb}$ in AMV-induced leukemogenesis, the unique ability of AMV to transform specifically only a discrete population of myeloid hematopoietic cells must be accounted for. Expression of nuclear p48$^{v\text{-}myb}$ in nonhematopoietic cells such as chicken fibroblasts (28) does not alter the morphology or the normal replication of these cells, indicating that cellular components specifically present in myeloid target cells are required for transformation. We propose, therefore, that the AMV oncogene product contains at least three functional domains that govern its leukemogenic properties: (i) a domain involved in interactions with lamina-nuclear matrix components, (ii) a domain involved in DNA binding, and (iii) a domain that determines its unique activity in myeloid stem cells. This last domain could be a recognition site activated by factors that specifically affect the replication or differentiation or both of myeloid cells, e.g., polypeptide hormones such as granulocyte-macrophage colony-stimulating factor (10) or interleukin 3 (23).

ACKNOWLEDGMENTS

We thank Carlo and Giovannella Moscovici for providing the BM2 cell line. We also thank Frank McKeon and Marc Kirschner for supplying us with human antilamin antisera.

This study was supported by Public Health Service research grant CA 10197 and Institutional National Research Service Award CA 09030 (to W.J.B. and M.A.L.) from the National Cancer Institute.

LITERATURE CITED

1. **Abrams, H. D., L. R. Rohrschneider, and R. N. Eisenman.** 1982. Nuclear location of the putative transforming protein of avian myelocytomatosis virus. Cell **24**:427–439.
2. **Baluda, M. A., B. Perbal, K. E. Rushlow, and T. S. Papas.** 1983. Avian myeloblastosis virus: a model for the generation of viral oncogenes from potentially oncogenic cellular genetic elements. Folia Biol. (Prague) **29**:18–34.
3. **Berezney, R., and D. S. Coffey.** 1975. Nuclear protein matrix: association with newly synthesized DNA. Science **189**:291–293.
4. **Berezney, R., and D. S. Coffey.** 1977. Nuclear matrix: isolation and characterization of a framework structure from rat liver nuclei. J. Cell Biol. **73**:616–637.
5. **Bodnar, J. W., C. J. Jones, D. H. Coombs, G. D. Pearson, and D. C. Ward.** 1983. Proteins tightly bound to HeLa cell DNA at nuclear matrix attachment sites. Mol. Cell. Biol. **3**:1567–1579.
6. **Boyle, W. J., M. A. Lampert, J. S. Lipsick, and M. A. Baluda.** 1984. Avian myeloblastosis virus and E26 virus oncogene products are nuclear proteins. Proc. Natl. Acad. Sci. U.S.A. **81**:4265–4269.
7. **Boyle, W. J., J. S. Lipsick, E. P. Reddy, and M. A. Baluda.** 1983. Identification of the leukemogenic protein of avian myeloblastosis virus and of its normal cellular homologue. Proc. Natl. Acad. Sci. USA **80**:2834–2838.
8. **Buckler-White, A. J., G. W. Humphrey, and V. Pigiet.** 1980. Association of polyoma T antigen and DNA with the nuclear matrix from lytically infected 3T6 cells. Cell **22**:37–46.
9. **Bunte, T., I. Greiser-Wilke, P. Donner, and K. Moelling.** 1982. Association of *gag-myc* proteins from avian myelocytomatosis virus wild-type and mutant with chromatin. EMBO J. **1**:919–927.
10. **Burgess, A. W., J. Camakaris, and D. Metcalf.** 1977. Purification and properties of colony-stimulating factor from mouse lung-conditioned medium. J. Biol. Chem. **252**:1998–2003.
11. **Burnette, W. N.** 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecylsulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein-A. Anal. Biochem. **112**:195–203.
12. **Capco, D. G., K. M. Wan, and S. Penman.** 1982. The nuclear matrix: three-dimensional architecture and protein composition. Cell **29**:847–858.
13. **Ciejeck, E. M., M.-J. Tsai, and B. W. O'Malley.** 1983. Actively transcribed genes are associated with the nuclear matrix. Nature (London) **306**:607–609.
14. **Curran, T., A. D. Miller, L. Zokas, and I. M. Verma.** 1984. Viral and cellular *fos* proteins: a comparative analysis. Cell **36**:259–268.
15. **Donner, P., I. Greiser-Wilke, and K. Moelling.** 1982. Nuclear localization and DNA binding of the transforming gene product of avian myelocytomatosis virus. Nature (London) **296**:262–266.
16. **Downward, J., Y. Yardin, E. Mayes, G. Scrace, N. Totty, P. Stockwell, A. Ulrich, J. Schlessinger, and M. D. Waterfield.** 1984. Close similarity of epidermal growth factor receptor and v-*erb*-B oncogene protein sequences. Nature (London) **307**:521–527.
17. **Eisenman, R. N., C. Y. Tachibana, H. D. Abrams, and S. R. Hann.** 1985. v-*myc*- and c-*myc*-encoded proteins are associated with the nuclear matrix. Mol. Cell. Biol. **5**:114–126.
18. **Feldman, L. T., and J. R. Nevins.** 1983. Localization of the adenovirus E1A$_a$ protein, a positive-acting transcriptional factor, in infected cells. Mol. Cell. Biol. **3**:829–838.
19. **Gerace, L., and G. Blobel.** 1980. The nuclear envelope lamina is reversibly depolymerized during mitosis. Cell **19**:277–287.

20. **Gerace, L., A. Blum, and G. Blobel.** 1978. Immunocytochemical localization of the major polypeptides of the nuclear pore complex-lamina fraction. Interphase and mitotic distribution. J. Cell Biol. **79**:546–566.
21. **Hunter, T.** 1984. The proteins of oncogenes. Sci. Am. **251**:70–79.
22. **Hunter, T., and B. Sefton.** 1980. Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. Proc. Natl. Acad. Sci. USA **77**:1311–1315.
23. **Ihle, J. N., J. Keller, L. Henderson, F. Klein, and E. Palaszynski.** 1982. Procedures for the purification of interleukin-3 to homogeneity. J. Immunol. **129**:2431–2436.
24. **Johnson, D. A., J. W. Gautsch, J. R. Sportsman, and J. H. Elder.** 1984. Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. Gene Anal. Techn. **1**:3–8.
25. **Klempnauer, K.-H., and Bishop, J. M.** 1983. Transduction of c-*myb* into avian myeloblastosis virus: locating points of recombination within the cellular gene. J. Virol. **48**:565–572.
26. **Klempnauer, K.-H., T. J. Gonda, and J. M. Bishop.** 1982. Nucleotide sequence of the retroviral leukemia v-*myb* gene and its cellular progenitor c-*myb*: the architecture of a truncated oncogene. Cell **31**:453–463.
27. **Klempnauer, K.-H., G. Ramsay, J. M. Bishop, M. G. Moscovici, C. Moscovici, J. P. McGrath, and A. D. Levinson.** 1983. The product of the retroviral transforming gene v-*myb* is a truncated version of the protein encoded by the cellular oncogene c-*myb*. Cell **33**:345–355.
28. **Klempnauer, K.-H., G. Symonds, G. I. Evans, and J. M. Bishop.** 1984. Subcellular localization of proteins encoded by oncogenes of avian myeloblastosis virus and avian leukemia virus E26 and by the chicken c-*myb* gene. Cell **37**:537–547.
29. **Laemmli, U. K.** 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
30. **Lipsick, J. S., W. J. Boyle, M. A. Lampert, and M. A. Baluda.** 1984. The oncogene of avian myeloblastosis virus is an altered proto-oncogene, p. 143–151. *In* G. F. Vande Woude et al. (ed.), Cancer cells, vol. 2: Oncogenes and viral genes. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
31. **McKeon, F. D., D. L. Tuffanelli, K. Fukuyama, and M. W. Kirschner.** 1983. Autoimmune response directed against conserved determinants of nuclear envelope proteins in a patient with linear scleroderma. Proc. Natl. Acad. Sci. USA **80**:4374–4378.
32. **McKeon, F. D., D. L. Tuffanelli, S. Kobayashi, and M. W. Kirschner.** 1984. The redistribution of a conserved nuclear envelope protein during the cell cycle suggests a pathway for chromosome condensation. Cell **36**:83–92.
33. **Moelling, K., E. Pfaff, H. Beug, P. Beimling, T. Bunte, H. E. Schaller, and T. Graf.** 1985. DNA-binding activity is associated with purified *myb* proteins from AMV and E26 viruses and is temperature-sensitive for E26 *ts* mutants. Cell **40**:983–990.
34. **Mollenhauer, H. H.** 1964. Plastic embedding mixtures for use in electron microscopy. Stain Technol. **39**:111–114.
35. **Moscovici, M. G., and C. Moscovici.** 1980. AMV-induced transformation of hemopoietic cells: growth patterns of producers and nonproducers, p. 503–514. *In* G. B. Rossi (ed.), *In vivo* and *in vitro* erythropoiesis: the Friend system. Elsevier, New York.
36. **Perbal, B., J. M. Cline, R. L. Hillyard, and M. A. Baluda.** 1983. Organization of chicken DNA sequences homologous to the transforming gene of avian myeloblastosis virus. II. Isolation and characterization of λ proto-*amv* DNA recombinant clones from a library of leukemic chicken DNA. J. Virol. **45**:925–940.
37. **Perbal, B., J. S. Lipsick, J. Svoboda, R. F. Silva, and M. A. Baluda.** 1985. Biologically active proviral clone of myeloblastosis-associated virus type 1: implications for the genesis of avian myeloblastosis virus. J. Virol. **56**:240–244.
38. **Quinlan, M. P., L. B. Chen, and D. M. Knipe.** 1984. The intranuclear location of a herpes simplex virus DNA-binding protein is determined by the status of viral DNA replication. Cell **36**:857–868.
39. **Razin, S. V., V. V. Chernoklavostov, A. V. Roodyn, I. B. Zbarsky, and G. P. Georgiev.** 1981. Proteins tightly bound to DNA in the regions of DNA attachment to the skeletal structures of interphase nuclei and metaphase chromosomes. Cell **27**:65–73.
40. **Rushlow, K. E., J. A. Lautenberger, T. S. Papas, M. A. Baluda, B. Perbal, J. G. Chirikjian, and E. P. Reddy.** 1982. Nucleotide sequence of the transforming gene of avian myeloblastosis virus. Science **216**:1421–1423.
41. **Shaper, J. H., D. M. Pardoll, S. H. Kauffmann, E. R. Barrack, B. Vogelstein, and D. S. Coffey.** 1979. The relationship of the nuclear matrix to cellular structure and function. Adv. Enzyme Regul. **17**:213–248.
42. **Smith, H. C., and R. Berezney.** 1982. Nuclear matrix-bound deoxynucleic acid synthesis: an *in vitro* system. Biochemistry **21**:6751–6761.
43. **Smith, H. C., E. Puvion, L. A. Buchholtz, and R. Berezney.** 1984. Spatial distribution of DNA loop attachment and replication sites in the nuclear matrix. J Cell Biol. **99**:1794–1802.
44. **Souza, L. M., J. N. Strommer, R. L. Hillyard, M. C. Komaromy, and M. A. Baluda.** 1980. Cellular sequences are present in the presumptive avian myeloblastosis virus genome. Proc. Natl. Acad. Sci. USA **77**:5177–5181.
45. **Staufenbiel, M., and W. Deppert.** 1983. Different structural systems of the nucleus are targets for SV40 large T antigen. Cell **33**:173–181.
46. **Toda, T., I. Uno, T. Ishikawa, S. Powers, T. Katoaka, D. Broek, S. Cameron, J. Broach, K. Matsumoto, and M. Wigler.** 1985. In yeast, *ras* proteins are controlling elements of adenylate cyclase. Cell **40**:27–36.
47. **Waterfield, M. D., G. T. Serace, N. Whittle, P. Stroobant, A. Johnson, A. Wasteson, B. Westermark, C.-H. Heldin, J. S. Huang, and T. F. Deuel.** 1983. Platelet-derived growth factor is structurally related to the putative transforming protein in p28^{sis} of simian sarcoma virus. Nature (London) **304**:35–39.
48. **Watt, R. A., A. R. Shatzman, and M. Rosenberg.** 1985. Expression and characterization of the human c-*myc* DNA-binding protein. Mol. Cell. Biol. **5**:448–546.