MOLECULAR AND CELLULAR BIOLOGY, Jan. 1985, p. 114–126
0270-7306/85/010114-13$02.00/0

Vol. 5, No. 1

v-*myc*- and c-*myc*-Encoded Proteins Are Associated with the Nuclear Matrix

ROBERT N. EISENMAN,* CHRISTINE Y. TACHIBANA,† HOLLY D. ABRAMS,‡ AND STEPHEN R. HANN

Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104

Received 27 July 1984/Accepted 9 October 1984

A series of extraction procedures were applied to avian nuclei which allowed us to define three types of association of v-*myc*- and c-*myc*-encoded proteins with nuclei: (i) a major fraction (60 to 90%) which is retained in DNA- and RNA-depleted nuclei after low- and high-salt extraction, (ii) a small fraction (1%) released during nuclease digestion of DNA in intact nuclei in the presence of low-salt buffer, and (iii) a fraction of *myc* protein (<10%) extractable with salt or detergents and found to have affinity for both single- and double-stranded DNA. Immunofluorescence analysis with anti-*myc* peptide sera on cells extracted sequentially with nucleases and salts confirmed the idea that *myc* proteins were associated with a complex residual nuclear structure (matrix-lamin fraction) which also contained avian nuclear lamin protein. Dispersal of *myc* proteins into the cytoplasm was found to occur during mitosis. Both c-*myc* and v-*myc* proteins were associated with the matrix-lamin, suggesting that the function of *myc* may relate to nuclear structural organization.

Recent evidence indicates that the proteins encoded by retroviral oncogenes fall into two classes with respect to their predominant subcellular localizations. The proteins encoded by the rather diverse oncogene families showing various degrees of relatedness to *src* or *ras* have been known for some time to be present in the cytoplasmic or plasma membrane compartments or in both. Recently it has been shown that the protein products of the *myc*, *myb*, and *fos* genes have a nuclear localization (37). Although gross subcellular location provides only a hint as to oncogene function, the nuclear localization of *myc* proteins (1, 4, 16, 23) is especially intriguing in light of evidence suggesting that *myc* may relate to cell growth control (10, 28, 32, 38). In addition it appears that alterations at the *myc* locus may be underlying events in many lymphoid and at least several nonlymphoid neoplasms in birds, rodents, and humans. The role that *myc* plays in these events, however, is unknown.

In principle, three classes of *myc*-related proteins can be considered: the v-*myc*-encoded proteins produced by the avian acute leukemia viruses; the c-*myc* protein synthesized after translocation, amplification, or promoter insertion in the vicinity of the c-*myc* allele; and the c-*myc* protein produced by an unaltered normal c-*myc* gene. In the avian system the group of acute leukemia viruses which possess the *myc* oncogene synthesize their v-*myc* proteins as either fusion products containing viral structural protein regions (usually derived from the *gag* gene which specifies the internal structural protein precursor) linked to v-*myc* protein regions (MC29 P110$^{gag\text{-}myc}$, CMII P90$^{gag\text{-}myc}$, OK10 P200$^{gag\text{-}pol\text{-}myc}$) or as v-*myc* proteins apparently not linked to any other viral proteins (OK10 p62$^{v\text{-}myc}$; MH2 p61/63$^{v\text{-}myc}$) (4, 23, 26). In avian bursal lymphoma cell lines, in which c-*myc* transcription has been increased due to proximal integration of a retroviral long terminal repeat, a 62,000-dalton protein has been identified as the lymphoma c-*myc* product (p62$^{c\text{-}myc}$) (4, 23). The proteins encoded by the c-*myc* gene in normal cells have proven more difficult to study due to the very low levels produced. However, using an anti-C-terminal *myc* peptide serum, we have recently identified two proteins in normal human (p64/p67) and avian (p59/p62) cells which are encoded by c-*myc* (25). All of these proteins are nuclear phosphoproteins.

As a means of elucidating the function of the *myc*-encoded proteins, we have studied the nature of their association with avian nuclei. In this paper we report the results of different extraction procedures on nuclei from fibroblast cells transformed by v-*myc*-containing retroviruses, as well as from bursal lymphoma cell lines containing high levels of c-*myc* protein. Our studies suggest that both *gag*-linked and non-*gag*-linked v-*myc* proteins as well as lymphoma c-*myc* proteins have multiple associations with nuclei, the predominant one being a very tight binding to a proteinaceous nuclear substructure (nuclear matrix).

MATERIALS AND METHODS

Cell lines. The following virus-transformed quail cell lines were used: MC29-transformed nonproducer quail cell line Q8 (obtained from K. Bister and recently subcloned), OK10 nonproducer quail cells (from M. Hayman), and HBC13 producer quail cells (from M. Hayman and recently subcloned). These lines were grown in Ham F-10 medium supplemented with 10% tryptose phosphate broth–5% calf serum–2% chick serum–0.5 to 1% dimethyl sulfoxide (DMSO). The RP9 cell line (LSCC-RP9) provided by W. Okazaki was established from a RAV-2-induced bursal lymphoma (31) and grown in Dulbecco modified Eagle medium with 5% calf serum–10% tryptose phosphate broth–1% chick serum–1 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid).

Immune assays. The rabbit anti-*gag* serum (5202-2), which was prepared against disrupted avian myeloblastosis virus, reacted only with avian retroviral *gag* proteins (see reference 17). Mouse monoclonal antibodies against avian lamin protein were a gift from P. Lu. The anti-v-*myc* 12C is an affinity-purified rabbit antibody prepared against a synthetic peptide corresponding to the 12 carboxy-terminal amino acids of the MC29 v-*myc* region. Both the preparation of this antibody and the *myc*-related proteins it recognizes have

* Corresponding author.
† Present address: Department of Biochemistry, Cellular and Molecular Biology, Cornell University, Ithaca, NY 14853.
‡ Present address: Clinical Division, Bio-Rad Laboratories, Richmond, CA 94804.

been described (23). For experiments involving competitive inhibition of antibody binding (blocking), 20 μg of peptide v-*myc* 12C per 10 μg of affinity-purified anti-v-*myc* 12C was mixed and incubated for 30 min at 4°C and used directly for precipitation or blotting.

Radioimmunoprecipitations were carried out as previously described (17) with the indicated rabbit antiserum and Formalin-fixed *Staphylococcus aureus*. Final washed pellets were dissolved in 5% sodium dodecyl sulfate (SDS) electrophoresis sample buffer, treated at 100°C for 3 min, and analyzed on SDS-polyacrylamide slab gels. Gels were prepared for fluorography by treatment with PPO (2,5-diphenyloxazole) in acetic acid. Immunoblot analysis was essentially by the method of Towbin et al. (43) with some modifications as described by Hann et al. (24). Proteins were detected by using the indicated rabbit antiserum (10 μg/ml for anti-v-*myc* 12C), followed by incubation with [^{125}I]protein A. Dried gels and blots were exposed to Kodak X-Omat R X-ray film.

Preparation of nuclei and nuclear extracts. (i) NP-40 nuclei. For DNase I, micrococcal nuclease, and RNase A treatment of nuclei (experiment described in the legend to Fig. 4), we prepared nuclei by first swelling whole cells, previously labeled with [^{3}H]thymidine for 16 h, in RSB hypotonic buffer (3 mM $MgCl_2$, 10 mM NaCl, 10 mM Tris-chloride [pH 7.4]). The swollen cells were then Dounce homogenized (tight A-type pestle) in RSB with 0.5% Nonidet P-40 (NP-40) until nuclei were free of obvious cytoplasmic contamination as judged by phase-contrast microscopy (see Fig. 9A). Nuclei were pelleted (350 to 2,000 rpm, 10 min; IEC PR6000 centrifuge) and suspended in RSB and the desired concentration of nuclease. Incubations were at 37°C for 10 min. Nuclei were then pelleted at 4°C at 12,500 × *g*, suspended in 5% SDS electrophoresis sample buffer, boiled for 3 min, and electrophoresed on SDS-polyacrylamide slab gels. A portion of the sample was removed before electrophoresis to determine the level of incorporated [^{3}H]thymidine by trichloroacetic acid (TCA) precipitation.

(ii) Further purification of nuclei (experiment described in the legend to Fig. 5). To obtain more stringently purified nuclei, we adopted the procedure of Thomas et al. (42). NP-40 nuclei were prepared as described above, suspended in 100 μl of NC buffer (40% glycerol, 2.5 mM $MgCl_2$, 50 mM Tris-chloride [pH 8.0]), mixed with 3 ml of TEN buffer (10 mM Tris-chloride [pH 8.0], 1.5 mM EDTA, 0.3 M NaCl), and incubated for 30 min at 4°C. The nuclei were then centrifuged through NC buffer, taken up in TMN (120 mM Tris-chloride [pH 7.4], 2 mM $MgCl_2$, 15 mM NaCl), and sonicated for six 10-s intervals at setting 3 (Branson sonifier with microprobe) at 0°C. This preparation could then be treated with nuclease (see Fig. 5A and B).

(iii) Preparation of residual nuclei (experiments described in the legends to Fig. 6 through 9). For preparation of residual nuclei, a modified procedure for preparing highly purified nuclei was used (from the method of Abrams et al. [1]). Whole cells were swelled in lysis buffer (5 mM KCl, 1 mM $MgCl_2$, 20 mM HEPES [pH 7.1], 0.5% aprotinin, and 0.1 mM PMSF [phenylmethylsulfonyl fluoride]) for 10 min at 4°C. Cells were Dounce homogenized with 25 strokes by using a tight fitting type A pestle in lysis buffer with 1% NP-40. After low speed centrifugation and suspension in lysis buffer containing 1% NP-40 and 0.5% deoxycholate, the cells were again homogenized until free of obvious contamination as determined by phase-contrast microscopy. These nuclei were then pelleted through a 1.8 M sucrose cushion, washed, and suspended in TMN with 1 mM PMSF. A portion of the sample was usually removed at this point to estimate nuclear protein. The remaining nuclei were incubated for 10 min at 37°C in TMN with 0.1 mM $CaCl_2$–100 μg of DNase I per ml–10 μg of RNase A per ml. DNase-treated nuclei were pelleted at 300 × *g* for 10 min. Pellets were washed at least twice in low-salt buffer (10 mM Tris-chloride [pH 7.4], 0.1 mM PMSF) and extracted twice for 15 min at 4°C in high-salt buffer (2 M NaCl, 10 mM Tris-chloride [pH 7.4], 0.2 mM $MgCl_2$, 0.1 mM PMSF). The final pellet from this step was suspended in SDS electrophoresis sample buffer and boiled as described above. Supernatants from the digestions and washes were collected, adjusted to 20% TCA, and incubated at 4°C for 4 h, and the precipitate was pelleted at 16,000 × *g* for 10 min. The pellets were washed once in ice-cold ethanol-ether (1:1) and once in ether alone and were finally suspended in SDS electrophoresis sample buffer and boiled before electrophoretic analysis.

DNA-cellulose chromatography. (i) LiCl extraction of nuclei. All operations were carried out at 4°C. NP-40 nuclei were prepared as described above. The nuclei were suspended in RSB containing 0.5% NP-40 and 0.5% aprotinin and dispersed by gentle homogenization, followed by centrifugation at low speed. The nuclear pellet was gently vortexed and extracted in a small volume of LiCl buffer (0.4 M LiCl, 10 mM Tris-hydrochloride [pH 8], 0.5 mM dithiothreitol [DTT], 0.5% aprotinin) with gentle rotation for 10 to 20 min, followed by centrifugation for 10 min at 10,000 × *g* to remove debris. The resultant supernatant was dialyzed against TE buffer (50 mM Tris-hydrochloride [pH 8], 2 mM EDTA) overnight, followed by an additional high-speed spin to remove any debris. Samples were stored at −80°C.

(ii) DNA-cellulose columns. Cellulose and denatured calf thymus DNA-cellulose were obtained from PL Biochemicals; native calf thymus DNA-cellulose was obtained from Sigma Chemical Co. Native quail cell DNA-cellulose was prepared by extracting DNA from Q8 cells, purifying the DNA, shearing the DNA by passing it 25 times through a 30-gauge needle, and then binding the DNA to cellulose by the drying and lyophilization method described by Alberts and Herrick (3); the quail cell DNA-cellulose obtained was at a concentration of 291 μg of DNA per packed ml of cellulose.

LiCl extracts from nuclei of various cell types were prepared and dialyzed. An equal volume of cold 2× loading buffer (0.03 M NaCl, 8 mM EDTA, 20% [wt/vol] glycerol, 1 mg of bovine serum albumin (BSA) per ml, 4 mM DTT [solution adjusted to pH 7.6]) was added to the extract before clarification by centrifugation at 10,000 × *g* for 10 min at 4°C. A sample of 0.4 to 0.8 ml was loaded on a 1-ml (packed volume) DNA-cellulose column; all operations were performed at 4°C. The procedure for binding to the DNA-cellulose columns was modified from that described by Alberts and Herrick (3). Elution buffers consisted of increasing concentrations of NaCl, 0.02 M Tris (pH 7.6), 5 mM EDTA, 10% (wt/vol) glycerol, 100 μg of BSA per ml, and 2 mM DTT. Elution buffer volumes were 2 ml per step.

For assay of extracts from Q8 cells, Q10H-transformed cells, or OK10-transformed cells, column fractions were precipitated with TCA, suspended in electrophoresis sample buffer, boiled, subjected to SDS-polyacrylamide gel electrophoresis, and analyzed by immunoblotting with either anti-*gag* serum (for Q8 and Q10H extracts) or anti-v-*myc* 12C peptide serum (for the OK10 extract). For assay of the extract from ^{32}P-labeled RP9 cells, column fractions were dialyzed against buffer containing 0.05 M NaCl, 0.02 M Tris-chloride (pH 7.4), and 0.25% aprotinin at 4°C overnight,

followed by the addition of detergents, immunoprecipitation with anti-v-*myc* 12C serum, electrophoresis, and autoradiography.

Immunofluorescence analysis of nuclear extractions. We followed the method described by Staufenbiel and Deppert (41) for immunofluorescence analysis of nuclear extractions. Briefly, cells were grown on 12-mm glass cover slips and first extracted in KM buffer (50 mM MES [morpholineethanesulfonic acid], 5 mM $MgCl_2$, 5 mM EGTA, 10 mM NaCl, 5 mM DTT), pH 6.0, supplemented with 0.5% NP-40 and 0.5% deoxycholate. The nuclear material remaining on the cover slips was then treated with KM buffer lacking DTT and EGTA and containing 50 μg of DNase I and 50 μg of RNase A per ml. The residual material remaining on the cover slip was washed by incubation with 25 mM KCl–40 mM Tris-chloride (pH 9.0)–2 M NaCl. Cover slips were collected for immunofluorescence and phase-contrast microscopy at each stage of the fractionation. Immunofluorescence was performed as described previously (1), using fixation in 1% formaldehyde and methanol at −20°C. Anti-v-*myc* 12C was used at a dilution of 1:30. Rhodamine-conjugated goat-anti-rabbit serum served as a second antibody and was used at a dilution of 1:4. 4′,6-Dianidino-2-phenylindole stain was used at a concentration of 10 μg/ml. Fluorescence was viewed on a Zeiss Photoscope III and photographed on Tri-X film (Kodak).

RESULTS

v-*myc* and c-*myc* proteins in high-salt extracts of avian nuclei bind to DNA. Previous studies on *myc*-encoded proteins have relied largely on immunoprecipitations with sonicated detergent extracts from different cells. To study in greater detail the association of *myc* proteins with the cell nucleus, we first examined the ability of different salts to extract v-*myc* protein from purified nuclei. The MC29-transformed quail cell line Q8 was labeled with [^{35}S]methionine and lysed in the presence of NP-40, and nuclei were prepared as described above. Under such detergent lysis conditions, only a very small fraction of *myc* protein can be found in the cytoplasmic fraction (1). Samples of the fresh nuclear fraction were incubated with 0.4 M LiCl, 2 M NaCl, 0.25 M $(NH_4)_2SO_4$, or 0.5% SDS. These salt concentrations are the same as those commonly used to extract a variety of nucleoplasmic proteins. The nuclei were pelleted, and the quantity of MC29 P110$^{gag\text{-}myc}$ extracted was determined by immunoprecipitation from the dialyzed supernatant and from the solubilized nuclear pellet. The results are shown in Fig. 1. The salt treatments led to removal of less than 10% of the v-*myc* protein from nuclei in this experiment. Only treatment with SDS resulted in extraction of a majority of the protein. Although we observed some variability in the absolute amount of *myc* protein eluted by salt treatment in different experiments (see, for example, Fig. 7A), the majority of the protein consistently appeared to be resistant to salt extraction. The DNA binding properties of v-*myc* and c-*myc* proteins in salt extracts are described below. In later sections we will show that the *myc* protein remaining associated with nuclei after salt extraction was present in an insoluble nuclear protein complex.

To determine whether the *myc* proteins in salt extracts of nuclei have affinity for DNA, we extracted purified Q8 cell nuclei with 0.4 M LiCl. The extract was dialyzed and passed through DNA-cellulose columns in low salt (3). Stepwise elutions were performed by using buffers with increasing concentrations of NaCl, and fractions were analyzed by gel electrophoresis and immunoblotting. A column containing

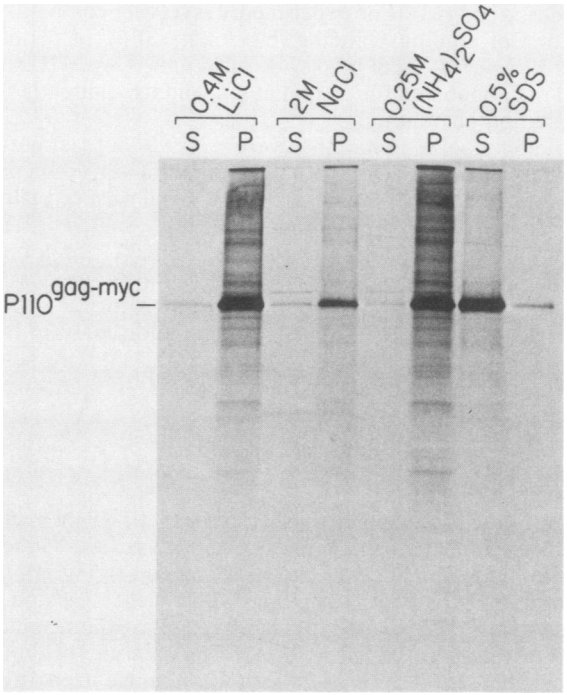

FIG. 1. Salt and detergent extractions of MC29 P110$^{gag\text{-}myc}$ protein from transformed quail cell nuclei. Three subconfluent 100-mm dishes of the MC29-transformed Q8 cell line were labeled for 2 h with 500 μCi of [^{35}S]methionine per dish, and nuclei were prepared after lysis in hypotonic buffer (RSB) containing 0.5% NP-40 as described in the text. Nuclei were divided into four samples and incubated in extraction buffer containing the indicated salt or 0.5% SDS for 20 min at 4°C with constant rotation. The samples were then centrifuged for 10 min at 10,000 × *g*. The nuclear pellets (lanes P) were suspended in extraction buffer, made 1% in Sarkosyl, freeze-thawed, and incubated at 100°C for 3 min. The supernatants (lanes S) were adjusted so that they contained equivalent amounts of detergent and were similarly treated. Both pellet and supernatant fractions were immunoprecipitated with anti-*gag* serum as described in the text. The resultant washed immunoprecipitates were boiled in 5% SDS electrophoresis sample buffer and electrophoresed on a 10% polyacrylamide gel. A fluorograph of the dried gel is shown. Lanes S, anti-*gag* immunoprecipitate of material extracted from nuclei; lanes P, anti-*gag* immunoprecipitate of material retained in nuclei after extraction.

cellulose without bound DNA would not retain the MC29 P110$^{gag\text{-}myc}$ protein (Fig. 2C). By contrast, columns containing cellulose linked to calf thymus single- or double-stranded DNA retained a high proportion of the P110$^{gag\text{-}myc}$ from salt-extracted Q8 cell nuclei. This material could be eluted with NaCl at concentrations ranging from 0.3 to 2.0 M (Fig. 2A and B). When the material present in the column flowthrough was pooled and chromatographed, most of the *myc* protein was found to bind to the DNA column (H. Abrams, unpublished data). This suggests that the presence of *myc* protein in the column flowthrough resulted from our having exceeded the capacity of the column rather than from a subpopulation of *myc* which did not bind DNA.

The retention of *myc* protein on the DNA-cellulose column did not appear to be dependent on the presence of *gag* sequences. This was shown by passing extracts from OK10-transformed quail cell nuclei (Fig. 3C) and from RP9 bursal lymphoma cell nuclei (Fig. 3B) over the DNA-cellulose column. In this experiment the DNA used was nondenatured

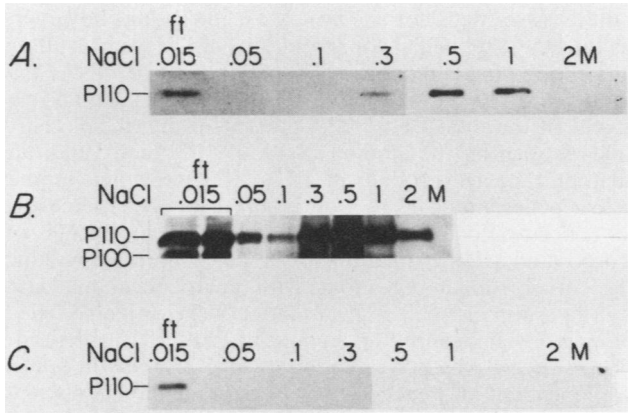

FIG. 2. DNA-cellulose chromatography of MC29 P110$^{gag\text{-}myc}$ in salt extracts of nuclei. As described in the text, Q8 nuclei were extracted with 0.4 M LiCl. In separate experiments the dialyzed lysates were passed through columns containing either (A) single- or (B) double-stranded calf thymus DNA linked to cellulose or (C) through cellulose alone. Samples were applied in buffer containing 0.015 M NaCl, and the columns were washed batchwise with buffers containing the indicated salt concentrations. Each wash fraction was precipitated with TCA, dissolved in SDS electrophoresis sample buffer, and electrophoresed on a 10% polyacrylamide gel. The proteins on the gel were electrophoretically transferred to a nitrocellulose sheet, and the sheet was probed with anti-*gag* serum and [^{125}I]protein A as described in the text. Only the relevant portions of the autoradiograph of the dried immunoblot are shown. ft, Flowthrough.

quail cell DNA. Both the OK10 p62$^{v\text{-}myc}$ and the lymphoma p62$^{c\text{-}myc}$ proteins were eluted from the DNA-cellulose column at 0.3 to 0.5 M NaCl. The proteins shown in Fig. 3 were specifically recognized by the antiserum, as indicated by the ability of the immunizing C-terminal peptide to block the immune precipitation of the 62-kilodalton protein from the bursal lymphoma cells (Fig. 3B, lanes b and i).

We also tested the relative DNA affinity of a protein containing an internal deletion in the *myc* region. The MC29 mutant, Q10H, fails to transform hematopoietic cells in vitro but has retained its ability to transform fibroblasts (7, 34). The data in Fig. 3A indicate that the P90$^{gag\text{-}myc}$ protein from Q10H nuclei was retained on the quail double-stranded DNA-cellulose column as tightly as was wild-type MC29 P110$^{gag\text{-}myc}$ when extracts from the two cell types were mixed. This suggests that the mutation had not blocked the potential for association with DNA. It is not clear, however, whether the apparently higher salt concentration required to elute P110$^{gag\text{-}myc}$ from the calf thymus DNA compared with the quail DNA (cf. Fig. 2A and B and Fig. 3A) actually reflected tighter binding to the former.

These data show that v-*myc* and c-*myc* proteins present in salt extracts from transformed cell nuclei could associate with DNA. Because the extracts were complex mixtures of nuclear proteins, it is possible that such binding was mediated through other proteins in the extract.

v-*myc* and c-*myc* proteins were retained in nuclei after DNase and RNase treatment. The results presented above indicate that v-*myc* and c-*myc* proteins obtained by high-salt extraction of nuclei were capable of binding to DNA. To determine whether *myc* protein association with the nucleus was dependent on intact DNA, we examined the distribution

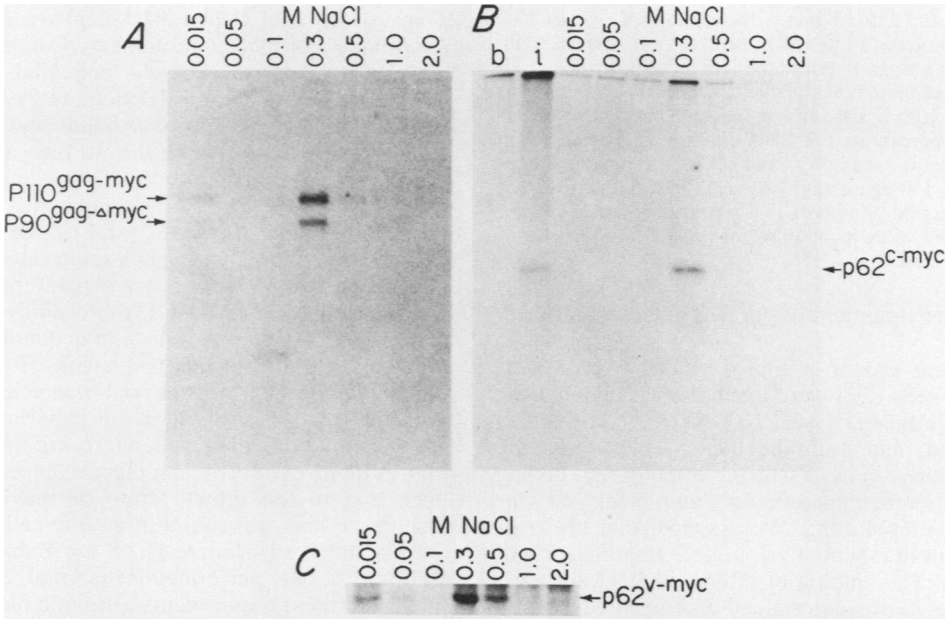

FIG. 3. DNA-cellulose chromatography of v-*myc*- and c-*myc*-encoded proteins in salt extracts of nuclei. Salt extracts (0.4 M LiCl) were prepared as described in the text. The dialyzed extracts were applied to a Q8 cell DNA-cellulose column in 0.015 M NaCl. The columns were eluted batchwise with the indicated salt concentrations. (A) Salt extracts prepared from Q8 cells and from Q10H quail cells. The extracts were mixed before DNA-cellulose chromatography. Fractions were analyzed as described in the legend to Fig. 1. An autoradiograph of the dried immunoblot is shown. (B) Extract prepared from the RP9 bursal lymphoma cell line labeled with $^{32}PO_4$ for 1 h. Fractions were analyzed by immunoprecipitation. Lane i, immunoprecipitate with anti-v-*myc* 12C antibody; lane b, immunoprecipitate with anti-v-*myc* 12C antibody preincubated with peptide v-*myc* 12C. An autoradiograph of the dried gel is shown. (C) Extract prepared from OK10-transformed quail cells. Fractions were treated and analyzed by electrophoresis and immunoblotting with anti-v-*myc* 12C. Only the relevant portion of the autoradiograph of the dried gel is shown.

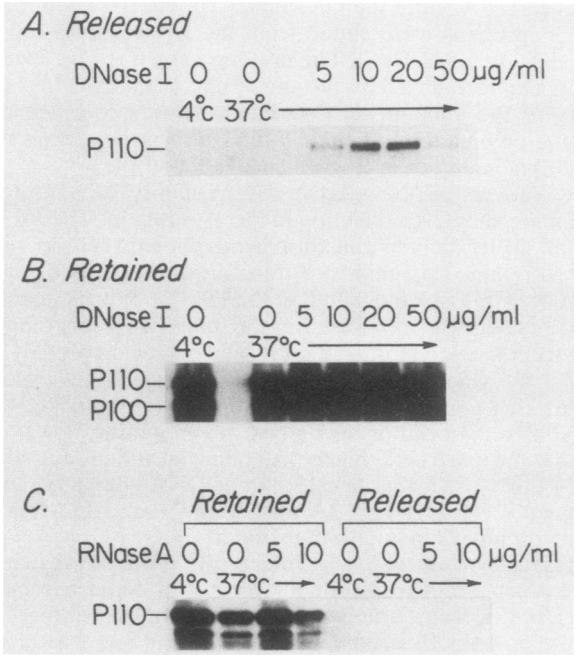

FIG. 4. Effects of DNase I and RNase A treatment on retention of MC29 P110$^{gag\text{-}myc}$ by Q8 nuclei. Three 150-mm dishes of subconfluent Q8 cells were swelled in hypotonic buffer, washed in the same buffer containing 0.5% NP-40, and lysed by Dounce homogenization. Lysis was checked by microscopy, and the low-speed nuclear pellet was suspended, divided into equal samples, and incubated with nuclease as indicated. After incubation, the nuclei were centrifuged at 12,800 × *g* for 5 min. The nuclear pellet retained (B) and the supernatant fraction released (A) were made 1× in electrophoresis sample buffer containing 5% SDS and 4% β-mercaptoethanol and were treated at 100°C for 3 min. Electrophoresis was on 10% polyacrylamide slab gels. P110$^{gag\text{-}myc}$ protein was detected by immunoblotting with anti-*gag* serum and iodinated protein A. Relevant portions of autoradiographs of the dried blots are shown. In parallel experiments with [^{3}H]thymidine-labeled Q8 cells, we determined the following percentages of DNA digested with DNase I by loss of TCA-precipitable radioactive material at the indicated concentrations of DNase I: 0 μg/ml, 0%; 5 μg/ml, 27%; 10 μg/ml, 8%; 20 μg/ml, 86%; and 50 μg/ml, 99.4%. (C) Of [^{3}H]uridine-labeled RNA, ca. 90% was digested after incubation for 10 min with 10 μg of RNase A per ml.

of *myc* protein after treatment of purified nuclei with nucleases.

In the experiment shown in Fig. 4, nuclei from MC29-transformed quail cells (Q8) were incubated with increasing concentrations of either DNase I or RNase A. The nuclei were then pelleted, and both the nuclear pellet and the supernatant fractions were dissolved directly by boiling in 5% SDS–4% β-mercaptoethanol and analyzed for P110$^{gag\text{-}myc}$ by immunoblotting. We assumed that this procedure would result in essentially complete solubilization of nuclear structures. The amount of DNA and RNA digested by nuclease treatment was determined by TCA precipitation of [^{3}H]thymidine- and [^{3}H]uridine-labeled samples. Incubation of nuclei without nuclease at either 4 or 37°C did not lead to release of the MC29 P110$^{gag\text{-}myc}$ protein into the nuclear supernatant (Fig. 4A). When the nuclei were incubated with DNase I at concentrations ranging from 5 μg/ml (27% DNA digested) to 50 μg/ml (99% DNA digested), increasing amounts of P110$^{gag\text{-}myc}$ could be detected in the nuclear supernatant. Loss of P110$^{gag\text{-}myc}$ from the supernatant at 50 μg/μl of DNase was due to protease contamination of the DNase (data not shown). Most intriguing, however, was the fact that even at concentrations of DNase I resulting in digestion of greater than 99% of the nuclear DNA, the quantity of P110$^{gag\text{-}myc}$ released represented less than a few percent of the total P110$^{gag\text{-}myc}$ present in the nuclei. This could be seen by the amount of P110$^{gag\text{-}myc}$ (and P100, an apparent cleavage product of P110$^{gag\text{-}myc}$) retained in the nuclear pellet after DNase I digestion (Fig. 4B). Increasing the concentration of DNase I to 100 μg/ml (0.05% of incorporated [^{3}H]thymidine counts per minute remaining [Fig. 5]) or similar digestions with micrococcal nuclease (75% digestion of labeled DNA [data not shown]) also failed to increase significantly the amount of P110$^{gag\text{-}myc}$ released.

To ascertain whether retention of *myc* proteins in nuclei was dependent on intact RNA, a sample of the Q8 nuclear preparation was incubated with RNase A. After incubation with 5 or 10 μg of RNase A per ml, essentially all of the P110$^{gag\text{-}myc}$ could be detected in the nuclear pellet with no detectable material released into the supernatant (Fig. 4C). At the latter concentration of RNase A, greater than 90% of the nuclear RNA was solubilized.

Our inability to detect the release of high levels of *myc* protein after nuclease digestion might have been due to the large size of the MC29 P110$^{gag\text{-}myc}$ protein, which may have prevented its diffusion out of intact nuclei. To test this possibility, we subjected Q8 nuclei to mild sonication until no intact nuclear structures were visible and then digested the preparation with 100 μg of DNase I per ml (>90% DNA digested in this experiment). Sonication did not lead to the release of P110$^{gag\text{-}myc}$ into the nuclear supernatant (Fig. 5A, lanes S), regardless of whether the sonic extract was treated with DNase I. Nearly all of the MC29 P110$^{gag\text{-}myc}$ protein was recovered in the sonicated nuclear pellet (Fig. 5A, lanes P).

***myc* proteins in DNA-, RNA-depleted nuclei.** Analysis of total major Q8 nuclear proteins by Coomassie blue staining of SDS-polyacrylamide gels indicated that DNase and RNase digestion of nuclei led to release of only a small subset of nuclear proteins (see below and Fig. 6, lanes 1 and 2). However, treatment with both high and low concentrations of salt after nuclease digestion resulted in extraction of essentially all of the histones and other groups of nuclear proteins (40; Fig. 6, lanes 5 and 6). To test whether *myc* protein association with nuclease-treated nuclei could be affected by salt extraction, we incubated nuclei from MC29 transformants with DNase I, followed by a 2 M NaCl wash of half the sample. A substantial quantity of P110$^{gag\text{-}myc}$ remained with the nuclear pellet (Fig. 5B, lanes P), although about one-third of the material was released in either the presence or absence of salt in this experiment (Fig. 5B, lanes S). The variability observed in terms of the absolute levels of *myc* protein extracted in different experiments may have been due to the growth state of the cells. An extreme example of this was found in mitotic cells (see below).

In a more stringent test of the nuclear association of P110$^{gag\text{-}myc}$, we performed sequential extractions on Q8 nuclei. In these experiments a sample of the nuclear preparation was left untreated for determination of total nuclear *myc* levels. The remaining nuclei were digested with both DNase I and RNase A, and the nuclear pellet was washed three times with low-salt buffer. Then the nuclear pellet from the low-salt washes was subjected to two 30-min extractions with 2 M NaCl. The supernatant fractions as well as the final pellet were then assayed for P110$^{gag\text{-}myc}$ by immunoblotting. The results (Fig. 5C) demonstrated that a significant amount of *myc* protein could be recovered in the final nuclear pellet.

Comparison with a sample of total untreated nuclei and direct assay for $P110^{gag\text{-}myc}$ in the postwash supernatants indicated that very little $P110^{gag\text{-}myc}$ was released in the high- and low-salt washes and that nearly all of the *myc* protein could be recovered in the final pellet (Fig. 5C). Thus, high-salt extraction either before or after nuclease digestion left most of the *myc* protein in the residual nuclear pellet, although a fraction of the protein was released. In other experiments, we varied the extraction procedure by performing digestions and washes at either pH 6.5 or 8.0, by using different buffer systems, or by carrying out the nuclease digestions without added 0.1 mM $CaCl_2$. None of these alterations in the extraction procedure had any substantial effect on the tight association of *myc* protein with nuclei (data not shown).

To determine whether c-*myc* and non-*gag*-linked v-*myc* proteins were as tightly associated with nuclei as was MC29 $P110^{gag\text{-}myc}$, we carried out a series of extractions on nuclei from the RP9 bursal lymphoma cell line and from OK10-transformed quail cells. The effects of different extraction procedures on the OK10 $p62^{v\text{-}myc}$ protein are shown in Fig. 7A. As was the case for $P110^{gag\text{-}myc}$, substantial amounts of the OK10 protein remained in the nuclear pellet after double detergent extraction, sonication, treatment at high pH, and high-salt extraction. We therefore repeated the sequential extractions with DNase I and RNase, followd by low-salt and extensive high-salt incubations and washes with RP9 and OK10-transformed cell nuclei. Immunoblots of the combined washes (Fig. 7B and C, lanes W) and residual nuclear pellets (Fig. 7B and C, lanes P) probed with anti-*myc* peptide serum (anti-v-*myc* 12C) are shown in Fig. 7B and C. Significant amounts of the OK10 $p62^{v\text{-}myc}$ and RP9 $p62^{c\text{-}myc}$ proteins remained in the final nuclear pellet (Fig. 7B and C, panels i). When the antiserum used in this immunoblot was preincubated with the C-terminal synthetic v-*myc* peptide used as immunogen, the p62 bands were no longer detected (Fig. 7B and C, panels b), indicating the specificity of the immune reaction.

Protein composition and structure of extracted nuclei. The effect of the extraction procedure used in the above experiments on Q8 total nuclear protein is shown in the Coomassie blue-stained gel in Fig. 6. Nuclease treatment alone released a group of nuclear proteins including histone H1, small amounts of other histones, and several higher-molecular-weight polypeptides (Fig. 6, lane 2). The low-salt and particularly the high-salt washes released another group of proteins including histone H1 and the core histones (Fig. 6, lanes 4 and 5), leaving the final nuclear pellet with a subset of proteins having a rather broad molecular weight range but marked by a nearly total absence of histones and other lower-molecular-weight species (Fig. 6, lane 6). The absence of histones and the digestion of 98% of the DNA argues for a quantitative extraction of chromatin under these conditions.

It was of interest whether the final nuclear pellet contained proteins known to constitute the residual nuclear structure known as the nuclear matrix-lamina. Therefore, we carried out an extraction of Q8 nuclei as described above, but in this instance the immunoblot was probed with a monoclonal antiserum against an avian lamin protein. The anti-lamin antibody recognized a single polypeptide in the 60,000- to 70,000-molecular-weight range (Fig. 8A). An indirect immunofluorescence assay with the anti-lamin serum on transformed quail cells is shown in Fig. 8B. Intense nuclear

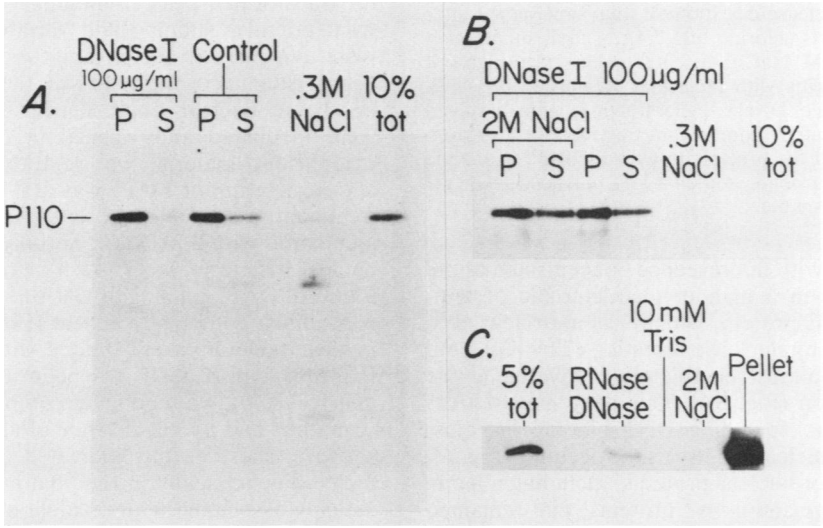

FIG. 5. Effects of different extraction procedures on nuclear retention of MC29 $P110^{gag\text{-}myc}$. Analyses shown were carried out by immunoblotting with anti-*gag* serum and iodinated protein A. Pellet fractions (P) were directly dissolved in electrophoresis sample buffer. Supernatant (S) and wash fractions were made 20% in TCA, and the protein precipitate was dissolved in sample buffer. In each experiment a fraction of the total nuclear preparation was removed and analyzed to estimate recovery (% tot). (A) NP-40 nuclei were prepared from three 100-mm dishes of Q8 cells. Nuclei were then incubated in NC buffer containing 0.3 M NaCl and pelleted through 40% glycerol. The suspended nuclear pellet was sonicated at maximum power for six 10-s intervals at 0°C. $CaCl_2$ was added to 0.1 mM, and the extract was divided into two samples. Sample 1 (DNase I) was incubated with 100 µg of DNase I per ml for 10 min at 37°C (90% digestion of DNA in this experiment). Sample 2 was incubated without nuclease (Control). Extracts were then centrifuged at 2,500 rpm (IEC PR6000 centrifuge) for 10 min to generate S and P fractions which were analyzed by immunoblotting. (B) Nuclei were purified, sonicated, and DNase I treated as in (A). One DNase I-treated sample was made 2 M in NaCl before final centrifugation. (C) Nuclei were prepared as described above except that Dounce homogenization was carried out in 1% NP-40–0.5% deoxycholate. Nuclei were pelleted through a cushion of 1.8 M sucrose, and the pellet was suspended in TMN buffer. DNase I (100 µg/ml) and RNase A (10 µg/ml) treatments were carried out as described above. The pelleted nuclei were then washed three times in 10 mM Tris-chloride buffer (pH 7.4) and twice, with 30-min incubations, in 2 M NaCl before the final pelleting of residual nuclei (Pellet).

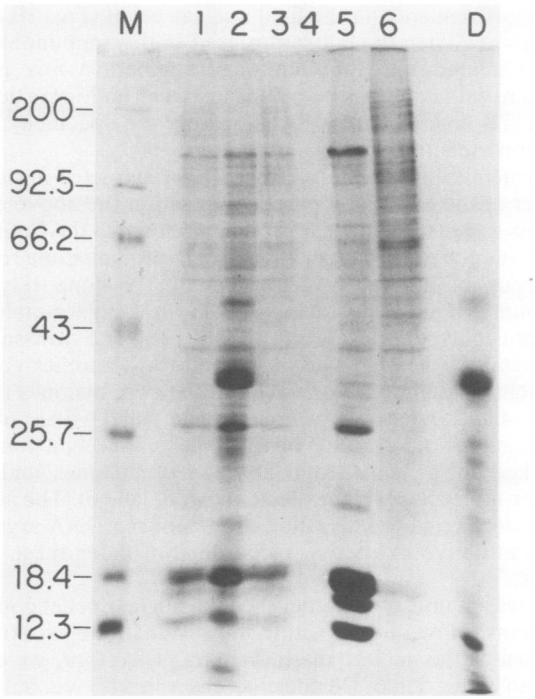

FIG. 6. Electrophoretic analysis of fractions from a sequential nuclear extraction: Coomassie blue staining. Nuclei from Q8 cells were prepared as described in the legend to Fig. 5C, followed by centrifugation through a sucrose cushion, suspension in TMN buffer, and treatment as indicated below to generate fractions representing released and retained material. Lanes: 1, 10% of the starting nuclear preparation; 2, the total supernatant after DNase I (100 μg/ml) and RNase A (10 μg/ml) treatment of nuclei; 3, 10% of nuclei remaining after nuclease digestion; 4, total supernatant after low-salt wash (10 mM Tris-chloride, pH 7.4); 5, total supernatant after high-salt wash (2 M NaCl); 6, total pellet after high-salt washes; M, marker proteins with molecular weights ($\times 10^3$) indicated to the left; D, 100 μg of DNase. Pellet fractions were dissolved directly in SDS sample buffer. Supernatant fractions were precipitated with 20% TCA, and the precipitate was dissolved in sample buffer. Samples were electrophoresed on a 15% polyacrylamide gel and stained with Coomassie blue.

staining was observed with fluorescence concentrated at the nuclear circumference in a manner characteristic of anti-lamin staining (22). This protein, which was not released to any appreciable extent by nuclease digestion or by high- and low-salt washes, was almost completely recovered in the residual nuclear fraction (Fig. 8A). Our data suggest that, whereas many proteins, including DNA-binding proteins, were released by the nuclease-salt wash procedure (Fig. 6), there existed a subset of nuclear proteins, including a lamin protein as well as v-*myc* and c-*myc* proteins, that remained associated with the residual nuclear pellet.

To ascertain whether any defined nuclear structure remained after the nuclease-salt wash procedure, we used phase-contrast microscopy to examine each of the pellet fractions resulting from the different extractions. Nuclei prepared as described above are shown in Fig. 9A and B. The nuclear envelope and prominent nucleoli are evident. Treatment with DNase I and RNase A resulted in a shrinkage of the nuclear structure and a loss of intranuclear material (Fig. 9C). After treatment of these nuclease-digested samples with low salt followed by 2 M NaCl, little change in the structure was observed, and residual nucleoli were still evident (Fig. 9D). When the nuclear structures in Fig. 9D were subjected to pressure by squeezing the cover slip, the structures became better spread, and some internal structure within a contiguous outer boundary could be observed (Fig. 9E). Thus, the final pellet obtained by our extraction procedure did not consist simply of amorphous nuclear debris but rather of material resembling residual nuclei.

Anti-*myc* immunofluorescence analysis of extracted nuclei. The phase-contrast microscopy analysis indicated that the major visual product of the nuclease-high-salt extraction of nuclei consisted of a structure resembling a residual nuclear skeleton. The v-*myc* and c-*myc* proteins detected by immunoblotting in the final pellet fraction could either be associated with this residual nuclear skeleton or present as part of the other debris evident in the phase-contrast assay (Fig. 9). To distinguish between these possibilities, we used anti-*myc* antibodies in an indirect immunofluorescence analysis of the nuclear material remaining after sequential extractions. We used the method outlined by Staufenbiel and Deppert (40, 41) in which cells were grown on glass cover slips, extracted in situ, and then fixed in formaldehyde and methanol. The cells were then incubated with specific rabbit antibody, and the bound antibody molecules were visualized by staining with rhodamine-labeled goat anti-rabbit immunoglobulin.

For the experiment shown in Fig. 10, we used quail cells transformed by HBC13, an MC29 variant (18) which, in our experiments, gave rise to somewhat flatter transformants than did MC29, thus facilitating the immunofluorescence analysis. Immunofluorescent staining of HBC13 cells with anti-v-*myc* 12C is shown in Fig. 10 with the corresponding phase-contrast image of the same field. Strong nuclear fluorescence was observed, as was demonstrated previously for transformed cells containing v-*myc* protein (23). Somewhat granular staining and variable fluorescence intensity were evident. All nuclei, however, showed visible fluorescence. Staining of the cells with the DNA-binding dye DAPI also gave nuclear fluorescence. When the transformants were incubated and washed in detergents, much of the cytoplasmic material was lost, but nuclear staining with anti-*myc* serum or DAPI was still observed (Fig. 10). After treatment with DNase I and RNase A and subsequent incubation with 2 M NaCl, anti-*myc* staining of the residual nuclear structures was still very evident, although somewhat reduced. DAPI staining of DNA in the nuclease-treated cells was almost completely absent (Fig. 10). When the residual nuclear skeletons were treated with the detergent Empigen BB, which previously has been used to solubilize nuclear matrices (40), we found a complete loss of the nuclear structures and a total absence of anti-*myc* staining (data not shown). These results indicated that the *myc* protein detected biochemically in the final nuclear pellet fraction was actually associated with residual nuclear structures.

Localization of *myc* protein in mitotic cells. To test the possibility that *myc* proteins might redistribute after nuclear dissolution during mitosis, we examined *myc* protein localization in colcemid-blocked mitotic cells. Growing cells were treated with colcemid, and the cells released by shakeoff after 5.5 h were cytofuged and examined by DAPI staining and immunofluorescence as described above. Cells having defined interphase nuclei, as determined by DAPI staining, also contained *myc* protein confined within the nuclear boundary (Fig. 11). One cell in this field, however, contained condensed chromatin characteristic of a premitotic cell (Fig. 11, arrow). In this cell the anti-*myc* staining was excluded from condensed chromatin and could then be seen to be

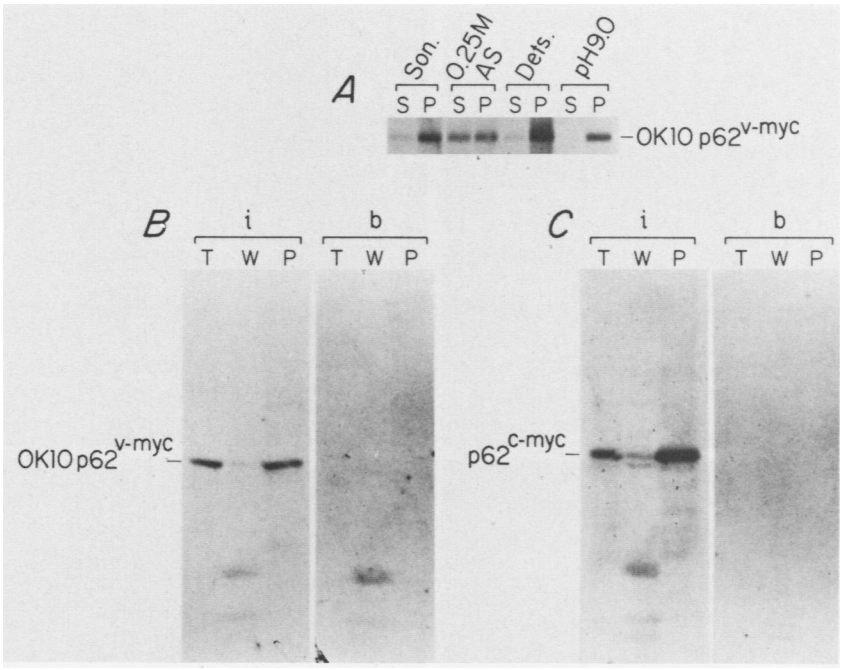

FIG. 7. Association of v-*myc* and c-*myc* proteins with residual nuclei. (A) Nuclei were prepared from OK10-transformed cells that had been labeled with [^{35}S]methionine for 5 h. Equal samples were sonicated twice for 10 s (Son.), extracted in 0.25 M ammonium sulfate (AS), treated with 1% Tween 80–0.5% deoxycholate and sheared through a 26-gauge syringe (Dets.), or incubated at pH 9 in STM (0.25 M sucrose, 50 mM Tris-chloride, 5 mM $MgCl_2$; pH 9.0) for 15 min. The pellets obtained after centrifugation were dissolved in 1% Sarkosyl, sonicated, heated for 3 min at 100°C, and diluted into buffer containing 0.5% SDS. The supernatant fraction was brought to the same concentrations of detergents, and both fractions were immunoprecipitated with anti-v-*myc* 12C. (B and C) Nuclei were prepared from OK10-transformed quail cells (B) and the RP9 bursal lymphoma cell line (C). Of the nuclear preparation, 20% was removed (lanes T). Remaining nuclei were treated first with DNase I-RNase A and then with 10 mM Tris–2 M NaCl as before, and the supernatants were combined for analysis (lanes W). Lanes P, final pellet fractions. All samples were analyzed by immunoblotting. Blots i, anti-v-*myc* 12C serum; blots b, anti-v-*myc* 12C serum preincubated with peptide v-*myc* 12C.

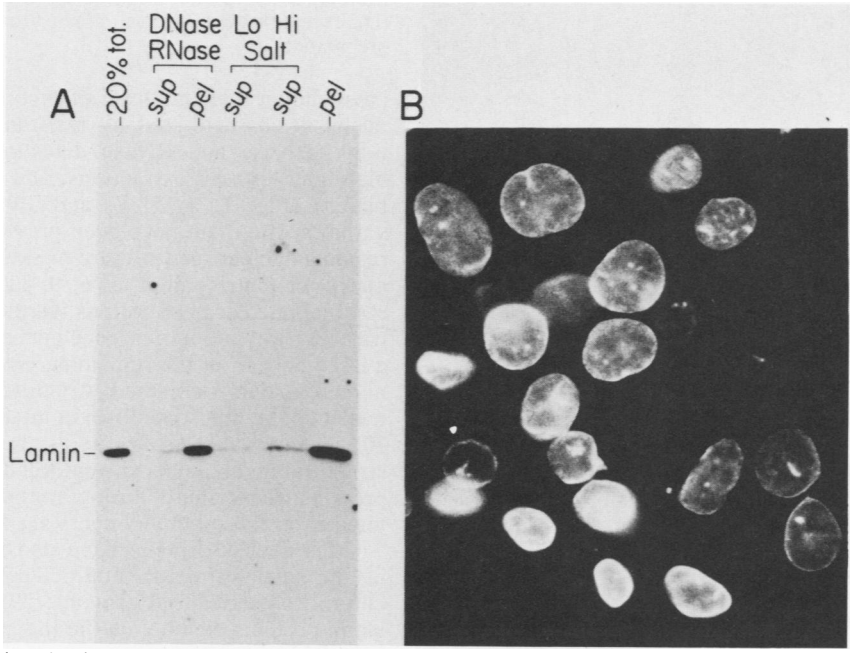

FIG. 8. Analysis of nuclear lamin protein in Q8 cells. (A) Immunoblot of nuclear fractions resulting from sequential extractions with nucleases and salts as described in the legend to Fig. 6. A monoclonal anti-lamin antibody was used for the blot. (B) Q8 cells grown on glass cover slips were fixed and stained by indirect immunofluorescence with the same monoclonal antibody used in (A) and with affinity-purified rhodamine-conjugated goat anti-mouse antibodies. Only nuclear fluorescence is observed.

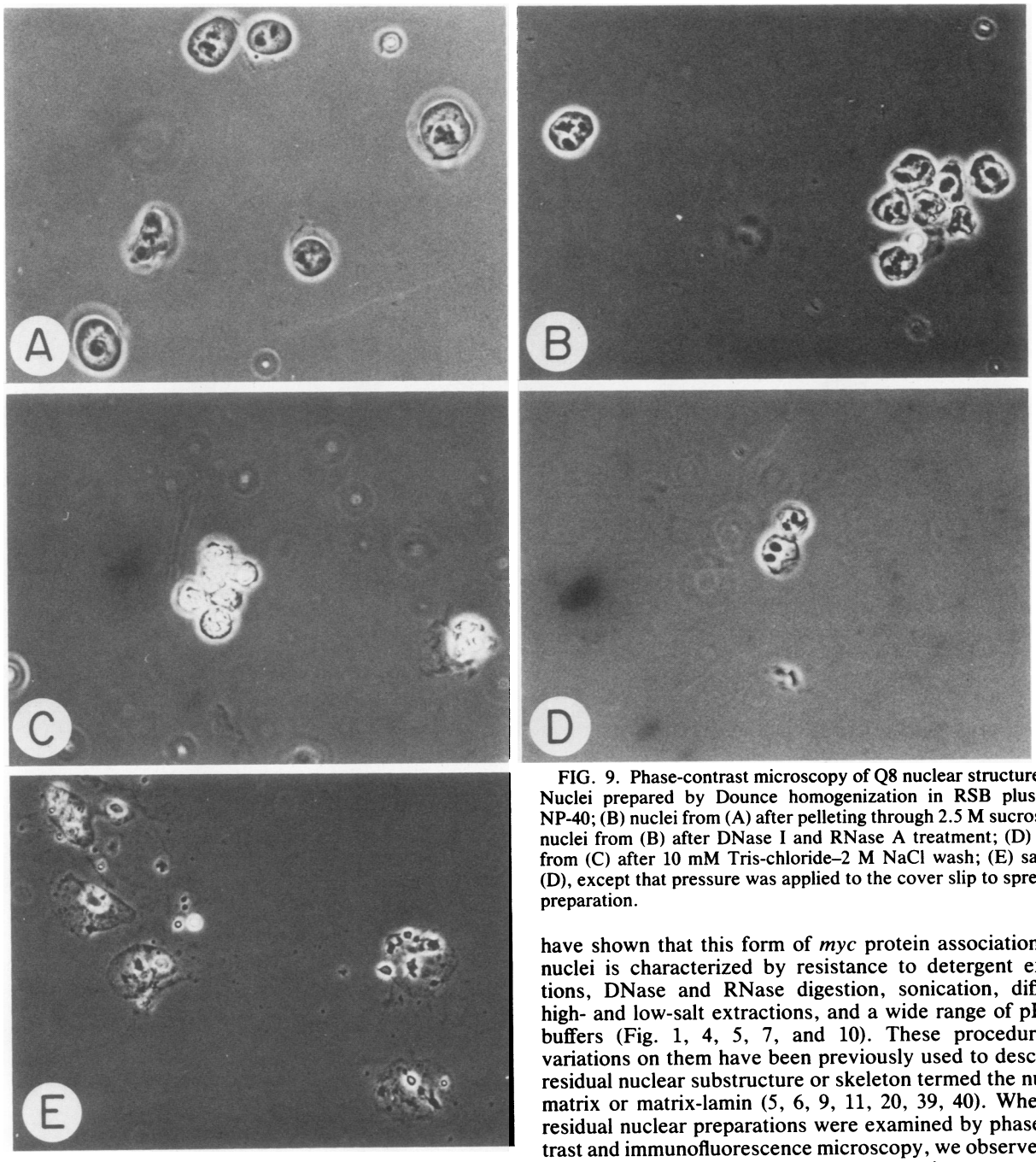

FIG. 9. Phase-contrast microscopy of Q8 nuclear structures. (A) Nuclei prepared by Dounce homogenization in RSB plus 0.5% NP-40; (B) nuclei from (A) after pelleting through 2.5 M sucrose; (C) nuclei from (B) after DNase I and RNase A treatment; (D) nuclei from (C) after 10 mM Tris-chloride–2 M NaCl wash; (E) same as (D), except that pressure was applied to the cover slip to spread the preparation.

dispersed throughout the cytoplasm. This more-generalized localization of *myc* protein was representative of mitotic cells in the population. In other experiments we clearly observed cytoplasmic *myc* fluorescence during mitosis of non-colcemid-treated cells (data not shown). This suggested that *myc* proteins changed their distribution during the cell cycle.

DISCUSSION

v-*myc* and c-*myc* proteins are associated with the nuclear matrix. The results described above demonstrate that most of the *myc* oncogene protein product is associated with nuclei in a manner independent of bulk nucleic acid. We have shown that this form of *myc* protein association with nuclei is characterized by resistance to detergent extractions, DNase and RNase digestion, sonication, different high- and low-salt extractions, and a wide range of pH and buffers (Fig. 1, 4, 5, 7, and 10). These procedures or variations on them have been previously used to describe a residual nuclear substructure or skeleton termed the nuclear matrix or matrix-lamin (5, 6, 9, 11, 20, 39, 40). When our residual nuclear preparations were examined by phase-contrast and immunofluorescence microscopy, we observed that a large portion of the remaining *myc* protein was present in numerous DNA-depleted structures resembling shrunken nuclei containing some form of internal structure (Fig. 9 and 10). The idea that *myc* proteins are associated with nuclear structural components is reinforced by the finding that *myc* proteins redistribute during mitosis, a time when other nuclear structural components are also dispersed (Fig. 11).

Other nuclear proteins have also been shown to be present in the nuclear matrix-lamin. These include the internal chromosomal scaffold proteins (29), the well-characterized lamin proteins which underlie the nuclear envelope (20–22), tight DNA-binding proteins (8, 35), and certain DNA tumor virus-transforming proteins, such as simian virus 40 and polyomavirus large T antigens (9, 40, 44), adenovirus E1A$_a$ (19), and herpesvirus ICP8 proteins (33). Our *myc*-containing

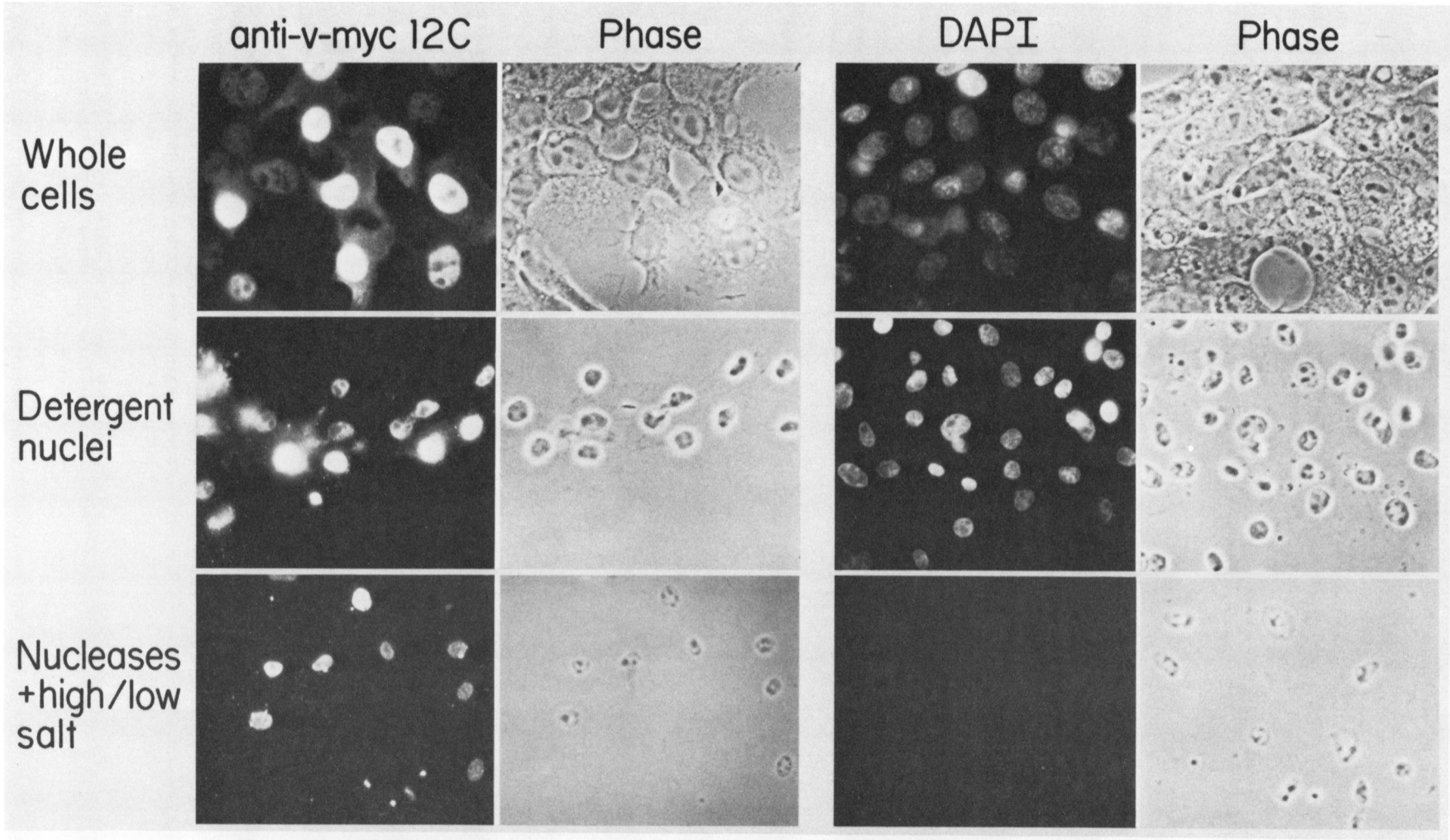

FIG. 10. Immunofluorescence analyses and phase-contrast micrographs of MC29-transformed quail cells and their nuclear structures extracted in situ. HBC13 cells were grown on glass cover slips. Parallel cover slips from the same culture were extracted in situ with NP-40 and deoxycholate (Detergent nuclei) or detergent extracted and then treated with DNase I and RNase A as described in the text. Cover slips from each extraction were stained with anti-v-*myc* 12C or with the DNA intercalating dye DAPI. Stained fields were also observed by phase-constrast microscopy. Results shown are from a single experiment.

residual nuclear preparations also appear to contain lamin protein, as determined by use of a monoclonal antibody prepared against one of the avian lamin proteins (Fig. 8). However, the distributions of lamin and *myc* protein in interphase nuclei were quite distinct, and comparison of the two immunofluorescence patterns suggests that the predominant location of *myc* is not the nuclear lamina (cf. Fig. 8 and 10). Biochemical separation of the internal matrix from the lamina (29) will be required to determine whether some subpopulation of the *myc* proteins is lamin associated. It is interesting that lamin proteins have also been shown to have affinity for DNA (29) and to disperse upon nuclear dissolution during mitosis (20, 21, 27).

The matrix-lamin localization of *myc* proteins may indicate that they are either part of the matrix structure itself or bound to protein, to nucleic acid components of the matrix structure, or to both. Recent kinetic studies of the c-*myc* protein in human cells (25) as well as previous studies on avian v-*myc* proteins (17; S. Hann, unpublished data) have shown a half-life of only 20 to 40 min. Such a short turnover time appears to be incompatible with *myc* proteins acting directly as structural elements. However, we do not know to what extent a dynamic or treadmilling type of structure may exist in nuclei. Another possibility is that *myc* encodes tight nucleic acid-binding proteins of the type demonstrated to associate with the DNA or RNA remaining after exhaustive nuclease digestion (8, 35). The association of *myc* protein with the matrix could also be mediated by interaction with other protein components. Our extraction procedures result in a residual nuclear structure that is nearly devoid of chromatin and contains a subset of mostly higher-molecular-weight polypeptides which includes lamin proteins (Fig. 6 and 8). We have not yet determined which, if any, of these residual proteins are associated with the *myc* product.

A population of less tightly bound *myc* protein. We have shown that extraction of intact nuclei with different salts, at concentrations generally used for extractions of the majority of simian virus 40 T antigen and nucleoplasmic proteins (11, 13), results in removal of 5 to 20% of the *myc* protein ($P110^{gag\text{-}myc}$ in Fig. 1). We believe that some of the variability observed may be due to changes in cell growth in different experiments. Small amounts of *myc* protein can also be extracted by double detergent wash or treatment at high pH (Fig. 7). The salt- and detergent-extracted protein probably represents a nucleoplasmic *myc* product that is loosely bound to structures anchored within nuclei, if at all. Most of the *myc* protein in such extracts has affinity for single- or double-stranded DNA (Fig. 2 and 3). This apparent affinity for DNA may be due to the binding of *myc* protein to DNA directly, or it may be mediated through other proteins in the extract. Other studies have shown that immunoaffinity-purified *gag-myc* protein is capable of tight binding to DNA (16), but it was unclear whether the harsh elution methods used in the latter studies led to denaturation of the protein. Indeed, we have detected lowered but significant binding of DNA by $P110^{gag\text{-}myc}$ after extended treatment of the extract at pH 2 (H. Abrams, unpublished data). Our studies show that both non-*gag*-linked v-*myc*- and c-*myc*-encoded proteins in nondenaturing salt extracts apparently have affinity for DNA. We have also found that a v-*myc*-encoded protein produced by an MC29 mutant containing an internal deletion in *myc* binds to DNA with the same apparent affinity as do wild-type *myc* proteins. A previous study (15) with filter binding of affinity-purified mutant *myc* protein detected lowered binding compared with that of wild-type protein. Since our analysis used salt extracts of quail fibroblasts which are transformed by this mutant (34), it is possible that the difference in the two results could be due to interaction with another component in the extract which mediates or stabilizes DNA binding by *myc* protein.

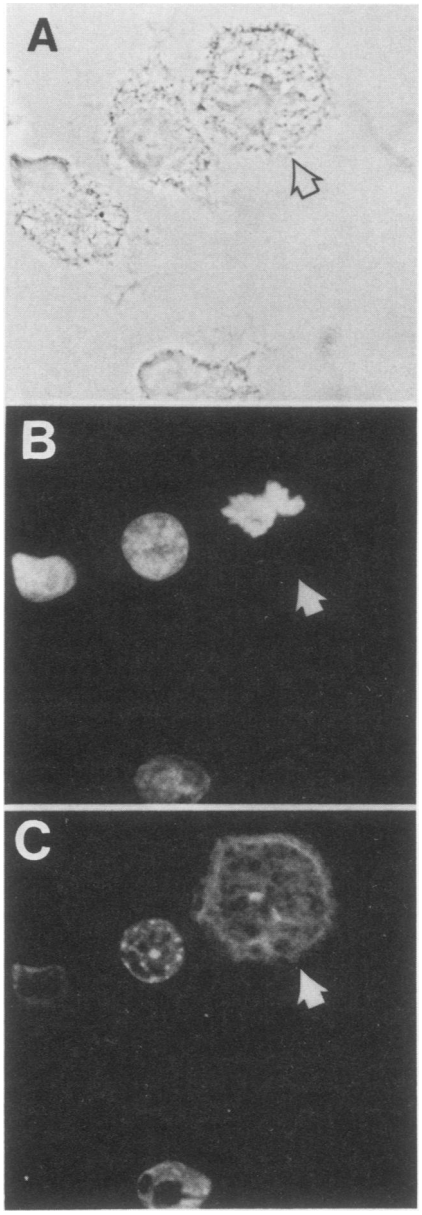

FIG. 11. Localization of *myc* proteins in mitotic cells. (A) Phase-contrast micrograph of cells; (B) cells stained with DAPI; (C) cells stained with anti-v-*myc* 12C. MC29-transformed quail cells (HBC13) were treated for 5.5 h with 0.5 μg of colcemid per ml. Cells released by shakeoff were cytofuged at 800 rpm for 15 min onto glass cover slips. The cover slips were treated with DAPI stain or anti-v-*myc* 12C followed by rhodamine-conjugated goat anti-rabbit serum as described in the legend to Fig. 10. Arrow, a premitotic cell.

Another possible form of association of *myc* protein with nuclei was observed after treatment of intact nuclei with DNase I or micrococcal nuclease under low-salt conditions. Digestion of nuclear DNA with nucleases was found to reproducibly lead to a release of both v-*myc* and c-*myc* proteins from nuclei. The amount of *myc* protein released

from nuclei was dependent on the nuclease concentration. Low amounts of *myc* protein could be detected after digestion of only 25% of nuclear DNA. However, the total amount released represented only a few percent of the total nuclear *myc* protein (Fig. 4, 5, and 7). This was observed even after digestion of up to greater than 99% of nuclear DNA. The results of these and the more stringent extractions indicate that most DNA sequences are not bound to *myc* protein, a finding also supported by anti-*myc* immunofluorescence analysis of dividing cells (Fig. 11; unpublished results).

Possible roles of *myc*. At present it is not possible to relate the different subnuclear localizations of *myc* proteins to normal *c-myc* or altered *c-myc-v-myc* functions. It has been shown that *myc* activation is tightly coupled to growth stimulation of quiescent cells and thus may be connected in some manner to the entry of the cells or their early passage through the G1 phase of the cell cycle (10, 28). The earlier demonstrations that v-*myc* transformants have a significantly higher growth rate (32, 38) also support the idea that *myc* function is related to growth control. We speculate that *myc* proteins may affect cell growth by their continual interaction with some form of dynamic nuclear structure which, in turn, is involved in programming the cell for proliferation. Some support for this idea may be provided from studies indicating an association between replicating DNA and the nuclear matrix (2, 5, 36) as well as recent experiments showing transit of the herpesvirus ICP8 protein between the nuclear matrix and replicating viral DNA (33).

Several lines of experimental evidence have suggested that transcription as well as DNA replication may be coupled to a nuclear skeletal substructure (see references 12, 14, and 30 for recent examples). Thus, the *myc* proteins might also act to regulate expression of other gene products, perhaps more directly involved in cell growth, either by altering their relationship to the nuclear matrix or by directly interacting with regulatory sequences. The latter possibility may be reflected by the apparent affinity of *myc* proteins for DNA (Fig. 2 and 3) and the release of *myc* proteins after digestion of only a small fraction of nuclear DNA at low DNase concentrations (Fig. 4). These latter conditions parallel those used to preferentially digest genes in an active chromatin conformation (45).

The finding that a protein associated with the nuclear matrix may have a function which can underlie the genesis of several diverse tumor types suggests a relationship between nuclear structure and transformation. The present challenge is, thus, to identify the specific elements that interact with *myc* proteins and to elucidate their role in cell growth processes.

ACKNOWLEDGMENTS

We thank John Burch, Wolfgang Deppert, Uli Laemmli, Peter Lu, and Larry Rohrschneider for advice, discussion, and reagents. We are grateful to John Burch and Robert Margolis for critical readings of the manuscript and to Deborah Nordquist for expert technical assistance.

This work was supported by Public Health Serivce grant CA 20525 from the National Cancer Institute to R.N.E., who is a Scholar of the Leukemia Society of America. S.R.H. is supported by a Public Health Service training grant from the National Institutes of Health, and H.D.A. is supported by the Lady Tata Memorial Trust.

LITERATURE CITED

1. **Abrams, H. D., L. R. Rohrschneider, and R. N. Eisenman.** 1982. Nuclear location of the putative tranforming protein of avian myelocytomatosis virus. Cell **29**:427–439.
2. **Aelen, J. M. A., R. J. G. Opstelten, and F. Wanka.** 1983. Organization of DNA replication in *Physarum polycephalum*. Attachment of origins of replicons and replication forks to the nuclear matrix. Nucleic Acids Res. **11**:1181–1195.
3. **Alberts, B., and G. Herrick.** 1971. DNA-cellulose chromatography. Methods Enzymol. **210**:198–217.
4. **Alitalo, K., G. Ramsay, J. M. Bishop, S. O. Pfeifer, W. W. Colby, and A. D. Levinson.** 1983. Identification of nuclear proteins encoded by viral and cellular *myc* oncogenes. Nature (London) **306**:274–277.
5. **Berezney, R., and D. S. Coffey.** 1975. Nuclear protein matrix: association with newly synthesized DNA. Science **189**:291–293.
6. **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.
7. **Bister, K., G. M. Ramsay, and M. J. Hayman.** 1982. Deletions within the tranformation-specific RNA sequences of acute leukemia virus MC29 give rise to partially-transformation-defective mutants. J. Virol. **41**:754–766.
8. **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.
9. **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.
10. **Campisi, J., H. E. Gray, A. B. Pardee, M. Dean, and G. E. Sonenshein.** 1984. Cell cycle control of c-*myc* but not c-*ras* expression is lost following chemical transformation. Cell **36**:241–247.
11. **Capco, D. G., K. M. Wan, and S. Penman.** 1982. The nuclear matrix: three-dimensional architecture and protein composition. Cell **29**:847–858.
12. **Ciejek, 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.
13. **Clark, R., D. P. Lane, and R. Tjian.** 1981. Use of monoclonal antibodies as probes of simian virus 40 T antigen ATPase activity. J. Biol. Chem. **256**:11854–11858.
14. **Cook, P. R., J. Lang, A. Hayday, L. Lania, M. Fried, D. J. Chiswell, and J. A. Wyke.** 1982. Active viral genes in transformed cells lie close to the nuclear cage. EMBO J. **1**:447–452.
15. **Donner, P., T. Bunte, I. Greiser-Wilke, and K. Moelling.** 1983. Decreased DNA-binding ability of purified transformation-specific proteins from deletion mutants of the acute avian leukemia virus MC29. Proc. Natl. Acad. Sci. U.S.A. **80**:2861–2865.
16. **Donner, P., I. Greiser-Wilke, and K. Moelling.** 1982. Nuclear localization of DNA binding of the transforming gene product of avian myelocytomatosis virus. Nature (London) **296**:262–266.
17. **Eisenman, R. N., M. Linial, M. Groudine, R. Shaikh, S. Brown, and P. E. Neiman.** 1980. Recombination in the avian oncoviruses as a model for the generation of defective transforming viruses. Cold Spring Harbor Symp. Quant. Biol. **44**:1235–1247.
18. **Enrietto, P. J., L. N. Payne, and M. J. Hayman.** 1984. A recovered avian myelocytomatosis virus that induces lymphomas in chickens: pathogenic properties and their molecular basis. Cell **35**:369–379.
19. **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.
20. **Fisher, P. A., M. Berrios, and G. Blobel.** 1982. Isolation and characterization of a proteinaceous subnuclear fraction composed of nuclear matrix, peripheral lamina, and nuclear pore complexes from embryos of *Drosophila melanogaster*. J. Cell Biol. **92**:674–686.
21. **Gerace, L., and G. Blobel.** 1980. The nuclear envelope lamina is reversibly depolymerized during mitosis. Cell **19**:277–287.
22. **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.
23. **Hann, S. R., H. D. Abrams, L. R. Rohrschneider, and R. N. Eisenman.** 1983. Proteins encoded by the v-*myc* and c-*myc*

oncogenes: identification and localization in acute leukemia virus transformants and bursal lymphoma cell lines. Cell **34**:789–798.
24. **Hann, S. R., M. A. Bender, and R. N. Eisenman.** 1984. Analysis and purification of v-*myc* proteins using anti-peptide antibodies, p. 181–188. *In* G. F. VandeWoude, A. J. Levine, W. C. Topp, and J. D. Watson (ed.), Cancer cells 2/ oncogenes and viral genes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Hann, S. R., and R. N. Eisenman.** 1984. Proteins encoded by the human c-*myc* oncogene: differential expression in neoplastic cells. Mol. Cell. Biol. **4**:2486–2497.
26. **Hayman, M. J.** 1983. Avian acute leukemia viruses. Curr. Top. Microbiol. Immunol. **98**:109–126.
27. **Jost, E., and R. T. Johnson.** 1981. Nuclear lamina assembly, synthesis and disaggregation during the cell cycle in synchronized HeLa cells. J. Cell Sci. **47**:25–53.
28. **Kelly, K., B. H. Cochran, C. D. Stiles, and P. Leder.** 1983. Cell-specific regulation of the c-*myc* gene by lymphocyte mitogens and platelet-derived growth factor. Cell **35**:603–610.
29. **Lebkowski, J. A., and U. K. Laemmli.** 1982. Non-histone proteins and long-range organization of HeLa interphase DNA. J. Mol. Biol. **156**:325–344.
30. **Mariman, E. C. M., C. A. G. van Eekelen, R. J. Reinders, A. J. M. Berns, and W. J. van Venrooij.** 1982. Adenovirus heterogeneous nuclear RNA is associated with the host nuclear matrix during splicing. J. Mol. Biol. **154**:103–119.
31. **Okazaki, W., R. L. Witter, C. Romero, N. Nazerian, J.-M. Sharma, A. Fadly, and D. Ewert.** 1980. Induction of lymphoid leukosis transplantable tumours and establishment of lymphoblastoid cell lines. Avian Pathol. **98**:311–329.
32. **Palmieri, S., P. Kahn, and T. Graf.** 1983. Quail embryo fibroblasts transformed by four v-*myc*-containing virus isolates show enhanced proliferation but are non-tumorigenic. EMBO J. **2**:2385–2389.
33. **Quinlan, M. P., L. B. Chen, and D. M. Knipe.** 1984. The intranuclear distribution of a Herpes simplex virus DNA-binding protein is determined by the status of viral DNA replication. Cell **36**:857–868.
34. **Ramsay, G., T. Graf, and M. J. Hayman.** 1980. Mutants of avian myelocytomatosis virus with smaller *gag* gene-related proteins have an altered transforming ability. Nature (London) **288**:170–172.
35. **Razin, S. V., V. V. Chernokhvostov, 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.
36. **Robinson, S. I., B. D. Nelkin, and B. Vogelstein.** 1982. The ovalbumin gene is associated with the nuclear matrix in chicken oviduct cells. Cell **28**:99–106.
37. **Rohrschneider, L. R., and L. E. Gentry.** 1984. Subcellular locations of retroviral transforming proteins define multiple mechanisms of transformation, p. 269–306. *In* G. Klein (ed.), Advances in viral oncology, vol. 4. Raven Press, N.Y.
38. **Royer-Pokora, B., H. Beug, M. Clavier, H.-J. Winkhardt, R. R. Friis, and T. Graf.** 1978. Transformation parameters in chicken fibroblasts transformed by AEV and MC29 avian leukemia viruses. Cell **13**:751–760.
39. **Shaper, J. H., D. W. Pardoll, S. H. Kauffmann, E. R. Barrack, B. Vogelstein, and D. S. Coffey.** 1978. The relationship of the nuclear matrix to cellular structure and function. Adv. Enzyme Regul. **17**:213–248.
40. **Staufenbiel, M., and W. Deppert.** 1983. Different structural sytems of the nucleus are targets for SV40 large T antigen. Cell **33**:173–181.
41. **Staufenbiel, M., and W. Deppert.** 1984. Preparation of nuclear matrices from cultured cells: subfractionation of nuclei in situ. J. Cell Biol. **98**:1886–1894.
42. **Thomas, P. S., J. H. Shepherd, E. R. Mulvihill, and R. D. Palmiter.** 1981. Isolation of a nuclear ribonucleoprotein fraction from chick oviduct containing ovalbumin messenger RNA sequences. J. Mol. Biol. **150**:143–166.
43. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. **76**:4350–4354.
44. **Verderame, M. F., D. S. Kohtz, and R. E. Pollack.** 1983. 94,000- and 100,000-molecular-weight simian virus 40 T-antigens are associated with the nuclear matrix in transformed and revertant mouse cells. J. Virol. **46**:575–583.
45. **Weintraub, H., and M. Groudine.** 1976. Chromosomal subunits in active genes have an altered conformation. Science **93**:848–858.