[CANCER RESEARCH 48, 1244-1251, March 1, 1988]

Identification and Characterization of a Human Proliferation-associated Nucleolar Antigen with a Molecular Weight of 120,000 Expressed in Early G_1 Phase[1]

James W. Freeman,[2] Rose K. Busch, Ferenc Gyorkey, Phyllis Gyorkey, Brenda E. Ross, and Harris Busch

Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77030

ABSTRACT

Tumor nucleoli were treated with polyclonal antisera to normal human tissue nucleoli to block some determinants common to tumor and normal tissue nucleoli. Immunization of mice with these immune complexes resulted in the development of a monoclonal antibody (FB2) to a novel M_r 120,000 nucleolar proliferation-associated antigen. By indirect immunofluorescence, antibody FB2 produced bright nucleolar staining in a variety of malignant tumors, including cancers of the breast, liver, gastrointestinal tract, genitourinary tract, blood, lymph system, lung, and brain. Although specific nucleolar immunofluorescence was not detectable in most normal tissues, it was detectable in some proliferating nonmalignant tissues including spermatogonia of the testes, ductal regions of hypertrophied prostates, and phytohemagglutinin-stimulated lymphocytes. The M_r 120,000 antigen was not detectable in 48-h serum-deprived HeLa cells but was readily detectable (within 30 min) following serum refeeding. The M_r 120,000 antigen was not detected in retinoic acid-treated HL-60 cells following morphological differentiation but was detectable in 48-h phytohemagglutinin-treated lymphocytes. These studies suggest that the M_r 120,000 antigen is a proliferation-associated antigen which plays a role in the early G_1 phase of the cell cycle.

INTRODUCTION

It has long been known that pleomorphism and hyperactivity of the nucleolus are major characteristics of cancer cells (1). These observations prompted studies to determine if tumor nucleoli possess components that are absent from nucleoli of normal cells. After a number of studies (2-7) using polyclonal antisera to tumor nucleoli demonstrated antigenic differences between tumor and normal tissue nucleoli, efforts were made to purify and characterize specific tumor-associated nucleolar antigens. Chan *et al.* (8-10) and Takahashi *et al.* (11) purified nucleolar proteins with molecular weights of 54,000, 61,000, and 68,000 from rat and human tumors that were not found in normal tissues. Two-dimensional isoelectrofocusing-SDS gels also showed (12) a number of differences as well as similarities between peptides of normal human tissue nucleoli and human tumor nucleoli.

Because of the lack of reproducibility of polyclonal antisera, monoclonal antibodies to human tumor nucleolar proteins were developed. Two nucleolar antigens, p145 and p40, were found to be associated with proliferating cells (13-15). The p145 and p40 nucleolar antigens were found in a broad range of human tumors but were not detectable in most normal resting tissues.

Studies by others (16-21) using human autoimmune sera detected nuclear and nucleolar antigens in tumor cells and normal growing cells that were not found in normal resting tissues. These antigens are referred to as PCNA.[3] Of these, the best characterized PCNA is a M_r 36,000 antigen termed "cyclin" (16, 17, 19). Cyclin was reported (17) to be synthesized during the G_1 to S phases of the cell cycle and translocated from the nucleoplasm to the nucleolus at a specific time point in S phase.

The nuclear oncogene products of c-*fos*, c-*myc*, and c-*myb* are transiently expressed during the cell cycle and are reported to be associated with cell proliferation (22-24). These onc gene products are expressed in quiescent cells stimulated to proliferate in response to growth factors and are elevated in some tumors. The roles of the nucleolar antigens, PCNAs, and nuclear oncogene products in cell proliferation are under study.

This study reports a monoclonal antibody which reacts with a novel nucleolar antigen (p120) associated with proliferating cells. Like nucleolar antigens p145 and p40, antigen p120 was detectable in a broad range of human tumors but not in most nonmalignant tissues. Antigen p120 was not detectable in growth-arrested cells but was expressed early in the G_1 phase.

MATERIALS AND METHODS

Antigen Preparation and Antibody Production. Antigen was prepared as previously described (25). For each injection (of 5 mice), nucleoli isolated from 5 g of HeLa cells were suspended in 1 ml of 0.01 M Tris-HCl, pH 7.2, containing 5 mM EDTA and placed in an ice bath for 1 h. To mask nontumor antigens, the unfolded nucleolar structures were treated with antinormal human liver antisera from two rabbits (10 µl of each antiserum) and allowed to tumble overnight at 4°C (25). The resulting immune complexes were used for injection of mice and monoclonal antibodies were developed and cloned as previously described (13).

Polyacrylamide Gel Electrophoresis and Immunoblotting. Analytical electrophoresis was performed on 7.5% polyacrylamide gels with 1% SDS according to Takacs (26). Whole HeLa cells or proteins extracted from isolated HeLa nucleoli were dissolved in Laemmli buffer and heated in a 100°C heating block for 5 min. The dissolved samples were loaded on the gel and electrophoresed for 2 to 3 h at 40 mA. Gels were stained with Coomassie blue or were transferred to nitrocellulose according to the method of Towbin *et al.* (27). Excess binding sites on the nitrocellulose were blocked in 7% chicken serum/3% bovine serum albumin in PBS. MAb FB2 was added as a 1:300 dilution of ascites in a 150 mM NaCl buffer containing 5 mM EDTA/50 mM Tris/0.25% gelatin/0.05% NP-40 at pH 7.4 and incubated for 2 h at room temperature on a rotary shaker. The nitrocellulose strips were washed for 30 min in the same buffer without antibody. A rabbit anti-mouse antibody (1 µl/ml; Cappel, Malvern, PA) was then added to the strips and incubated at room temperature for 1 h with shaking. The strips were then washed; radioiodinated Protein A (200,000 cpm/ml) was added and incubated at room temperature for 1 h. Excess Protein A was removed by washing for 2 h with 1.0 M NaCl buffer containing 5 mM EDTA/50 mM Tris/0.25% gelatin/0.4% *N*-lauroylsarcosine, pH 7.4. The strips were then dried and analyzed by autoradiography.

Immunofluorescence Localization of p120. Immunocytochemical localization of nucleolar antigen p120 identified by monoclonal antibody

Received 4/13/87; revised 8/10/87, 11/2/87; accepted 11/11/87.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by Cancer Research Center Grant CA-10893, P1, awarded by the National Cancer Institute, Department of Health and Human Services, Public Health Service; The DeBakey Medical Foundation; the Davidson Fund; the Pauline Sterne Wolff Memorial Foundation; the H. Leland Kaplan Cancer Research Endowment; the Linda and Ronnie Finger Cancer Research Endowment Fund; the William S. Farish Fund; and the Sally Laird Hitchcock Fund.

[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: PCNA, proliferating cell nuclear antigen; MAb, monoclonal antibody; FB2, a monoclonal antibody to a M_r 120,000 nucleolar antigen; PHA, phytohemagglutinin; SDS, sodium dodecyl sulfate; PBS, 0.01 M PO4/0.15 M NaCl, pH 7.4; NP-40, Nonidet P-40; DMEM, Dulbecco's modified Eagle's minimal essential medium; protein p120, M_r 120,000 protein.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/48/5/1244/2434853/cr0480051244.pdf by guest on 24 August 2022

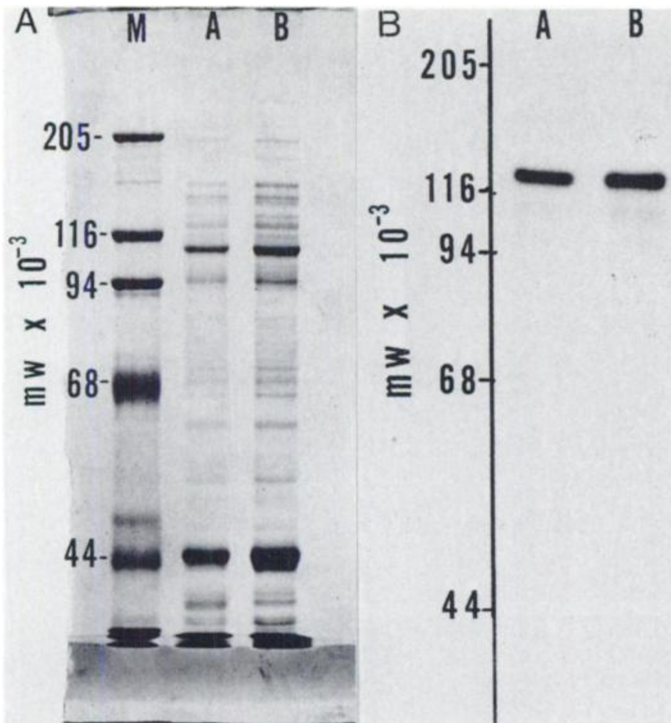


Fig. 1. *A*, Coomassie blue-stained gel of HeLa cell nucleolar proteins. *Lanes M*, molecular weight markers; *A*, 75 μg of protein, and *B*, 150 μg of protein. *B*, Western blot of two similarly loaded gel lanes as in *A* and stained with MAb FB2. Following incubation with primary antibody (FB2) the blots were incubated with a 1:1000 dilution of rabbit anti-mouse IgG and bands were detected by autoradiography after incubation with ^{125}I-labeled protein A (200,000 cpm/ml).

Table 1 *Immunoreactivity of a monoclonal antibody to nucleolar antigen p120 with human cancer tissues or cancer cells*

Specimen	No. of samples	Intensity of nucleolar immunofluorescence
Adenocarcinomas		
Primary: colon	2	+++
Primary: small bowel	1	+++
Metastatic: brain	1	++++
Carcinomas		
Primary		
hepatocellular	1	+++
lung	3	+++
nasopharyngeal	1	+++
cervical (squamous cell)	1	+++
prostate	2	+++
Metastatic		
skin	1	++++
lymph node	1	++++
Lymphomas (nonHodgkin's)	5	+++
Lymphoma (Hodgkin's)	1	+/−
Kaposi sarcoma	1	+++
Breast cancer: intraductal carcinomas	1	+++
	1	+/−
Leukemia (acute lymphocytic)	2	+++
Tumor cell lines		
HeLa	1	++
HL-60 (promyelocytic leukemia)	1	+++
Colon tumors		
Omega	1	+++
HCT	1	+++
Mosar	1	+++
JVC	1	+++
GEO	1	+++

Table 2 *Immunoreactivity of a monoclonal antibody to nucleolar antigen p120 with nonmalignant human tissues or cells*

Specimen	No. of specimens	Intensity of nucleolar immunofluorescence
Lymphocytes		
Untreated	1	−
PHA-treated (72 h)	1	++
Bone marrow	1	−
Kidney	3	−
Liver	3	−
Stomach	1	−
Gallbladder	2	−
Brain	2	−
Lymph node	2	−
Spleen	2	−
Breast	1	−
Lung	3	−
Colon	2	−
Mandibular tissue (inflamed)	1	−
Prostate[a]		
Hypertrophied	2	+ (1 of 2)
Normal	2	−
Testes	1	+/− trace

[a] Weak nucleolar immunofluorescence detectable in some ducts.

was detected by a modification of the indirect immunofluorescence method of Hilgers *et al.* (28). HeLa cells were grown on slides and fixed for 20 min with 2% formaldehyde in PBS. The cells were then permeabilized with ice cold acetone (20°C) for 3 min and stored in PBS. Cryostat sections of tumors or normal tissues were fixed and permeabilized in acetone for 10 min at −20°C. The primary antibody (anti-p120) was used at a dilution of 1:300 (ascites:PBS) which provided optimal immunofluorescence staining on control HeLa cell slides. A sufficient amount of diluted MAb (25 μl) was placed on cells or tissues and incubated in a humid atmosphere at 37°C for 1 h. The slides were washed twice for 15 min in PBS as above and mouse antibody was detected with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Boehringer Mannheim) diluted 1:50 in PBS.

Extraction of Nuclei and Nucleoli for Antigen Characterization. Nuclei and nucleoli were prepared by the NP-40 hypotonic shock method previously described (2). For nuclear proteins, nuclei from 10^8 cells were extracted by homogenation in lysis buffer (3 M urea/0.02% NP-40/0.05% mercaptoethanol). Laemmli buffer was added to the extract and boiled for 5 min and proteins were resolved by SDS polyacrylamide gel electrophoresis. Isolated HeLa nucleoli (0.3 g wet weight) were washed twice with 5 ml 0.075 M NaCl/0.025 M EDTA, pH 7.6, and extracted 4 times with 5 ml 10 mM Tris-HCl, pH 8.0/0.2% w/v sodium deoxycholate. Pellets were suspended using a Dounce homogenizer followed by centrifugation at 25,000 × *g* for 15 min. The Tris-deoxycholate extract was then treated with RNase overnight at 4°C (1000 units RNase T_1 and A) and then loaded on 5–45% sucrose density gradients in the same buffer. Other Tris-deoxycholate extracts not treated with RNase were separated on similar sucrose gradients. All extraction buffers contained 0.1 mM phenylmethylsulfonyl fluoride (Sigma, St. Louis, MO), 1 μg/ml leupeptin, and 1 μg/ml aprotinin (Boehringer Mannheim, Indianapolis, IN) to inhibit endogenous proteolytic activity.

Retinoic Acid-induced Differentiation of HL-60 Cells. The HL-60 human promyelocytic leukemia cell line was grown in suspension cultures in RPMI Medium 1640 (Grand Island Biological Co.) containing 10% fetal calf serum, 100 IU penicillin/ml, 100 μg streptomycin/ml, and 2 μM/ml *trans*-retinoic acid (Sigma) (14). HL-60 cells treated

Downloaded from http://aacrjournals.org/cancerres/article-pdf/48/5/1244/2434853/cr0480051244.pdf by guest on 24 August 2022

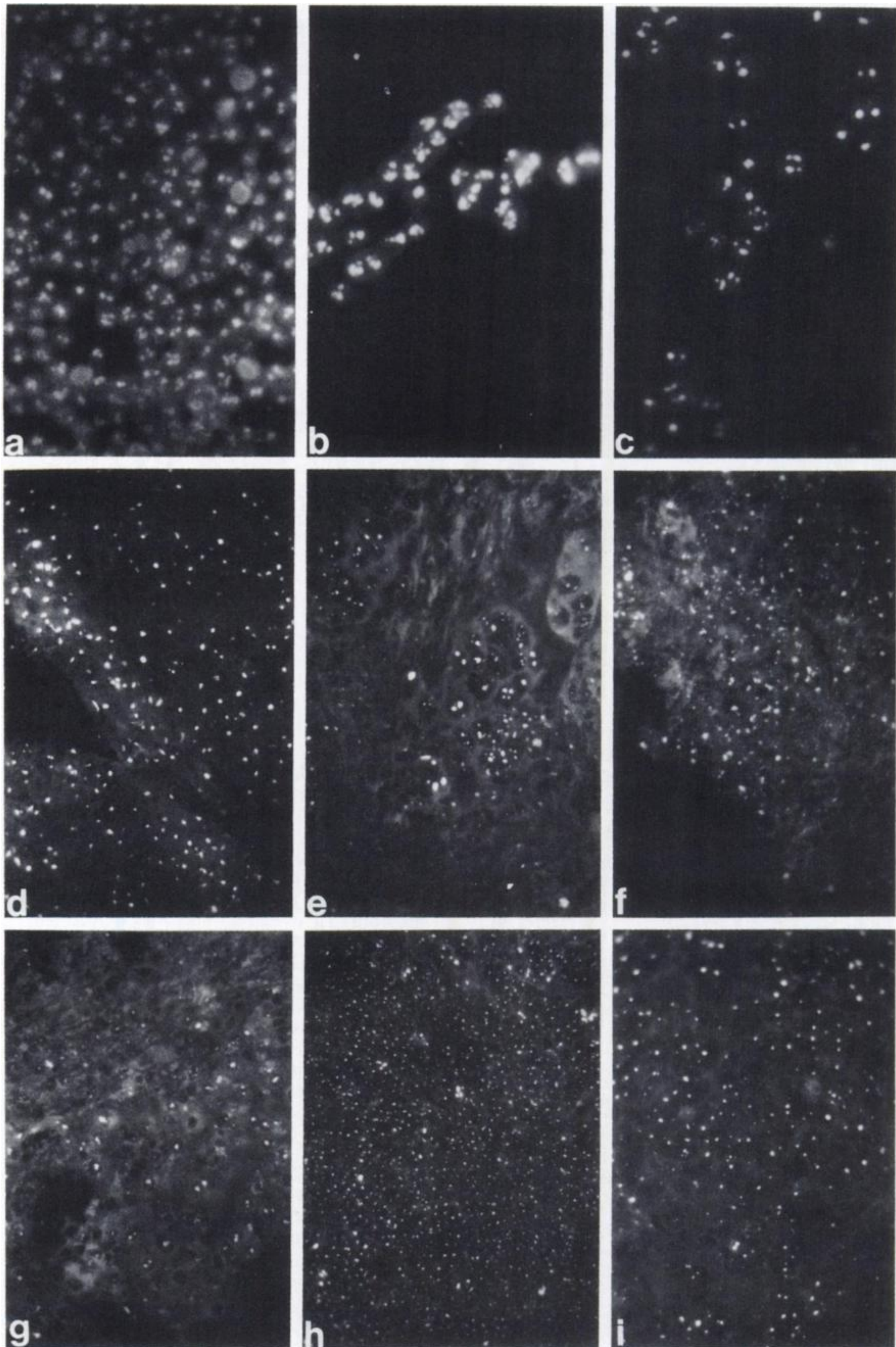


Fig. 2. Representative immunofluorescence detection of nucleolar antigen p120 with MAb FB2 in malignant tissues or cells. *a*, HeLa cells; *b*, Omega cells (human colon tumor cell line); *c*, HL-60 cells (human promyelocytic leukemia); *d*, squamous cell carcinoma; *e*, brain, adenocarcinoma; *f*, lung, carcinoma; *g*, liver, hepatocellular carcinoma; *h*, lymphoma; and *i*, Kaposi sarcoma. The primary MAb FB2 was added at a 1:300 dilution of ascites; for detection a goat anti-mouse IgG-fluorescein isothiocyanate diluted 1:50 was used.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/48/5/1244/2434853/cr0480051244.pdf by guest on 24 August 2022

with the retinoic acid were examined for specific immunofluorescence at 24-h intervals up to 120 h; the cells were attached to slides by cytocentrifugation. A side-by-side control of untreated HL-60 cells was done at each time.

Serum Deprivation Study. HeLa cells were grown for 48 h in DMEM containing 10% fetal calf serum in a humidified atmosphere with 5% CO_2. After 48 h, the medium was replaced by a DMEM which lacked the fetal calf serum and cultured for another 48 to 72 h. Following serum deprivation, the medium was replaced with fresh DMEM containing 10% fetal calf serum. Samples were collected hourly up to 7 h, except for the first hour when 30-min samples were collected; at each time the presence of p120 was determined by indirect immunofluorescence.

RESULTS

Monoclonal Antibody Development. The MAbs produced by immunization of mice with the immunomasked nucleoli identified several nucleolar antigens. Of these, one (FB2) was selected for further study because it showed preferential immunostaining of tumor tissues compared to normal tissues. The MAb FB2 identified a M_r 120,000 peptide on immunoblots of HeLa nucleolar proteins (Fig. 1). In our studies, trace bands of M_r 105,000 and 100,000 (Fig. 1*B*, *lane B*) were sometimes observed and these bands increased with storage of nucleolar extract with a corresponding decrease of the M_r 120,000 band suggesting that the minor bands represent degradation products.

Immunohistological Studies. The immunoreactivity of MAb FB2 to the nucleolar antigen p120 was examined by indirect immunofluoresence in a broad range of malignant and nonmalignant tissues and cells (Tables 1 and 2). Representative immunofluorescence patterns are shown in Figs. 2 and 3.

Bright nucleolar-specific fluorescence was detected in a broad range of malignant tumors and cells (Table 1; Fig. 2) including cancers of the gastrointestinal tract, genitourinary tract, liver, lung, breast, lymphatics, and blood. Nucleolar immunofluorescence was not detected in corresponding normal tissues (Table 2; Fig. 3). In some tissues (*i.e.*, kidney; Fig. 3) some general background staining was observed. This type of background fluorescence was also found in specimens to which no primary antibody was added.

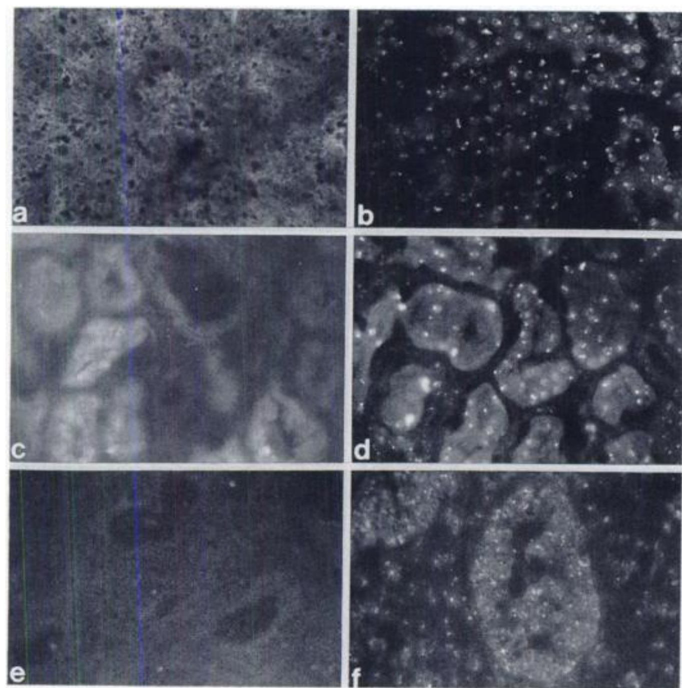


Fig. 3. Representative immunofluorescence of normal human tissues with MAb FB2 to antigen p120 and a positive control antibody to nucleolar protein B23. *a*, *c*, and *e*, liver, kidney, and colon, respectively, stained with the MAb FB2; and *b*, *d*, and *f*, corresponding sections stained with the control antibody to nucleolar protein B23 found in all tissues. The primary antibody was detected as in Fig. 2.

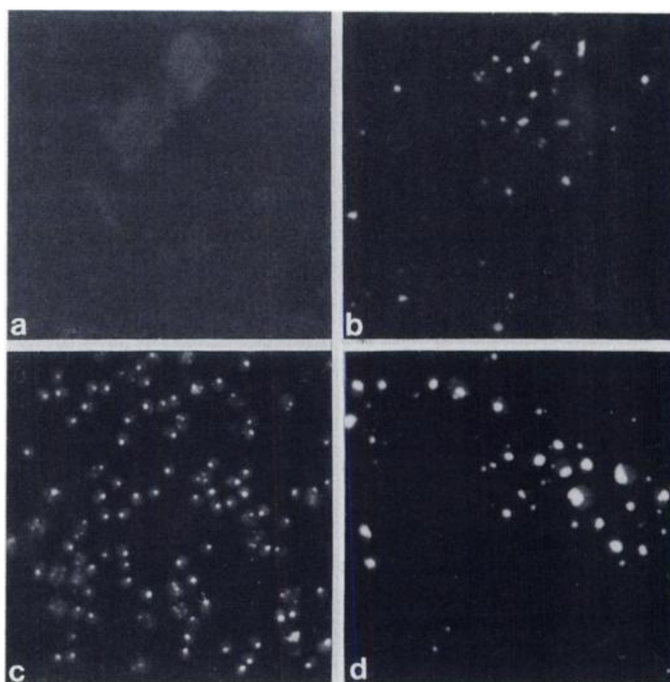


Fig. 4. Effect of PHA stimulation on the expression of nucleolar antigen p120 in human lymphocytes. *a* and *c*, untreated lymphocytes, and *b* and *d*, 48-h PHA-stimulated lymphocytes. *a* and *b* were reacted with MAb FB2 to antigen p120, and *c* and *d* were reacted with the MAb to protein B23. The primary antibodies were detected by immunofluorescence (see Fig. 2).

In most tumors, MAb FB2 produced bright nucleolar immunofluorescence. However, in one Hodgkin's lymphoma and one breast tumor only weak nucleolar fluorescence was observed. The lesser immunofluorescence intensity in these tumors may relate to type, staging, or treatment.

Nucleolar immunofluorescence was detectable in 48-h PHA-stimulated lymphocytes (Fig. 4*b*), spermatogonia of the testes, and the ductal epithelium of 1 of 2 hypertrophied prostates studied (Table 2).

The effect of cell proliferation on the expression of nucleolar antigen p120 was examined in (*a*) PHA-stimulated lymphocytes, (*b*) retinoic acid-induced differentiation of human leukemia HL-60 cells, and (*c*) HeLa cells whose growth was arrested by serum deprivation.

PHA Stimulation of Human Lymphocytes. Isolated human lymphocytes were treated in culture with PHA and examined by immunofluorescence at 24-h intervals for the detection of antigen p120 (Fig. 4). Nucleolar antigen p120 was not detectable in fresh peripheral blood lymphocytes nor in untreated lymphocytes maintained in cultures (Fig. 4*a*). The nucleolar structure was intact in these cells since anti-protein MAb B23 (protein B23 is a nucleolar antigen found in tumor and nontumor cells) produced bright nucleolar-specific fluorescence (Fig. 4*c*). The immunofluorescence for nucleolar antigen p120 reached maximum intensity in 48-h PHA-treated lymphocytes which had small dot-like immunofluorescence spots within the nucleolus (Fig. 4*b*). At this time, the morphology of the lymphocytes changed to that of blast cells. Immunostaining of 48-h PHA-treated lymphocytes with an anti-B23 MAb showed a much larger staining area within the nucleolus, with more

Downloaded from http://aacrjournals.org/cancerres/article-pdf/48/5/1244/2434853/cr0480051244.pdf by guest on 24 August 2022

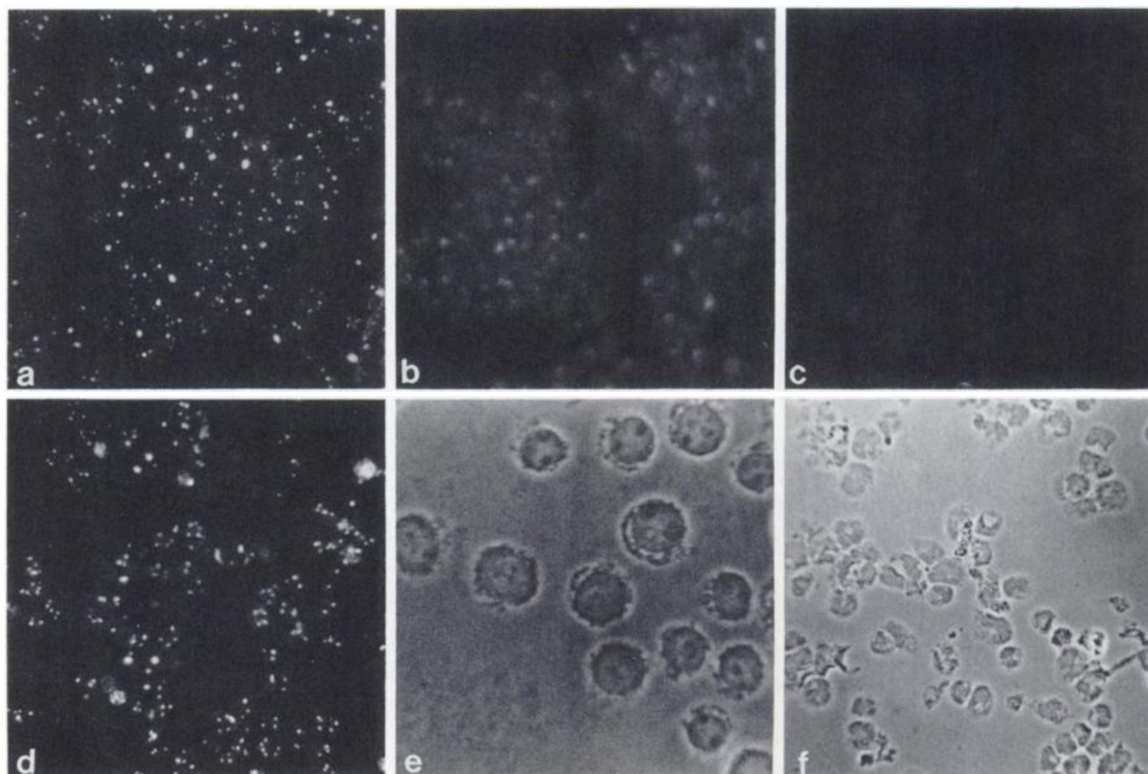


Fig. 5. Effect of retinoic acid-induced differentiation of HL-60 cells on the expression of nucleolar antigen p120. *a*, untreated; *b*, 48-h retinoic acid treatment; *c*, 96-h retinoic acid treatment; *d*, 96-h retinoic acid-treated cells reacted with control antibody to protein B23; *e*, morphology of untreated HL-60 cells (phase); and *f*, morphology of 96-h retinoic acid-treated cells. The cells were reacted with the MAb FB2 to nucleolar protein p120 or control MAb to protein B23; immunoreactivity was detected by immunofluorescence as in Fig. 2.

intense staining (Fig. 4*d*). Accordingly, p120 may be compartmentalized within the nucleolus.

HL-60 Cell Differentiation. As an obverse approach to mitogenic stimulation of resting lymphocytes, termination of cell proliferation was studied in retinoic acid-induced differentiation of human HL-60 cells. The retinoic acid-treated HL-60 cells approximately doubled in number up through 48 h; between 48 and 72 h a slight increase in cell number was found with no further increase in cell number between 72 and 96 h (not shown). Untreated control HL-60 cells showed bright nucleolar immunofluorescence staining with the MAb FB2 (Fig. 5*a*) and had a blast-like appearance (Fig. 5*e*). At 48 h of retinoic acid treatment of decrease in the immunofluorescence staining was observed (Fig. 5*b*). At 96 h, only low levels of immunofluorescence were observed (Fig. 5*c*). At this time, the cells had a more mature neutrophil-like appearance in retinoic acid-treated cultures (Fig. 5*f*). The nucleolar structure of differentiated cells was, however, maintained since all cells stained with the MAb to protein B23 (Fig. 5*d*). Antigen p120 expression decreased when cell cycling ceased.

Growth-arrested HeLa Cells. HeLa cells were growth arrested by 48 to 72 h of serum deprivation to determine whether antigen p120 is directly associated with cell cycling. In four separate experiments, decreased nucleolar immunofluorescence was found in 24-h serum-deprived HeLa cells. Detection of antigen p120 was lost between 36 and 72 h in serum-free media (Fig. 6*b*). Nucleolar fluorescence for protein B23 was positive throughout. Following serum replenishment antigen p120 was detected within 30 min (Fig. 6*c*) and the immunofluorescence increased to its original intensity by 2 h (Fig. 6*d*). Accordingly, antigen p120 is expressed in the early G_1 cell cycle phase.

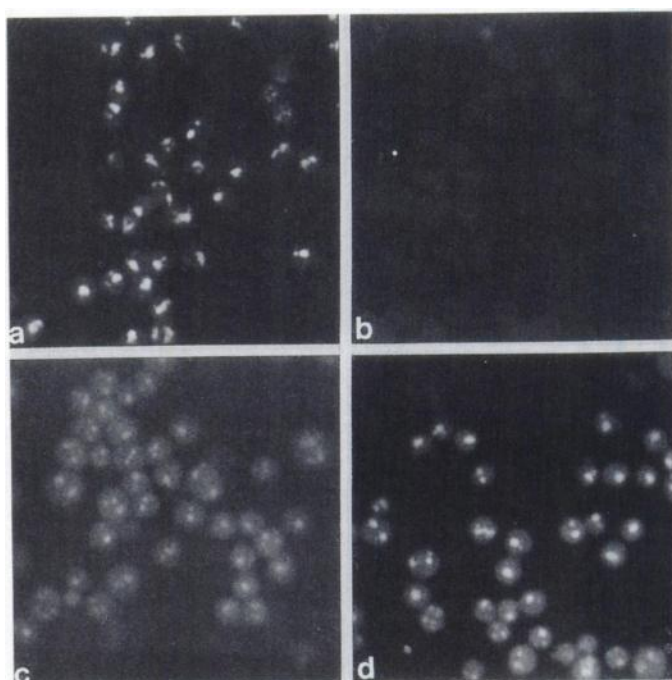


Fig. 6. Expression of antigen p120 following serum deprivation and serum replenishment in HeLa cells. *a*, control HeLa cells, 0 h time point; *b*, 48-h serum-deprived HeLa cells; *c*, 30 min after serum replacement; and *d*, 2 h after serum replacement. All cells were reacted with the MAb FB2 to nucleolar antigen p120; immunofluorescence was detected as noted in Fig. 2.

Immunoblotting Studies. It is possible that the lack of immunofluorescence staining observed in retinoic acid-treated HL-60 cells, in growth-arrested HeLa cells, and in nonproliferating normal tissues may represent a redistribution of the p120 antigen or inaccessibility of the p120 epitope. To investigate these possibilities, immunoblots were prepared of nuclei from

Downloaded from http://aacrjournals.org/cancerres/article-pdf/48/5/1244/2434853/cr0480051244.pdf by guest on 24 August 2022

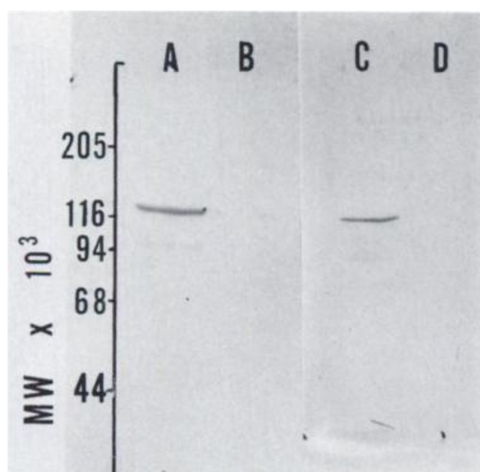
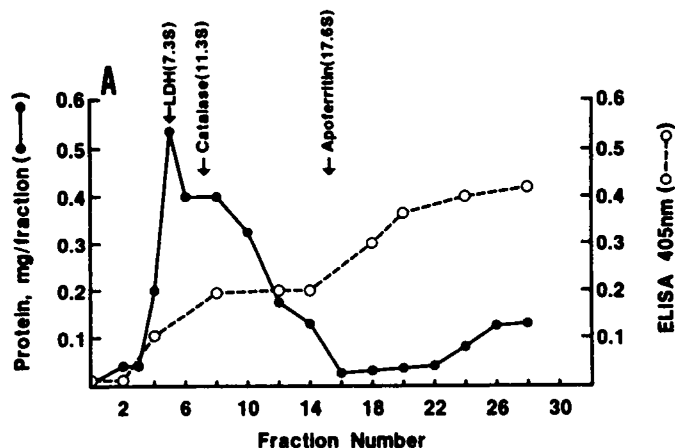


Fig. 7. Western blots of nuclear proteins from (*A*) untreated HL-60 cells, (*B*) retinoic acid-treated HL-60 cells, (*C*) serum-fed HeLa cells, and (*D*) serum-deprived HeLa cells. Nuclei were isolated according to "Materials and Methods" from equal numbers of cells (10^8). Nuclei were extracted in 3 M urea/0.02% NP-40/0.05% mercaptoethanol and an equal volume of 2× Laemmli buffer was added. Equal amounts of protein (75 μg) were loaded and electrophoresed on a 7.5% polyacrylamide gel. Antigen was detected following Western transfer as described in "Materials and Methods."



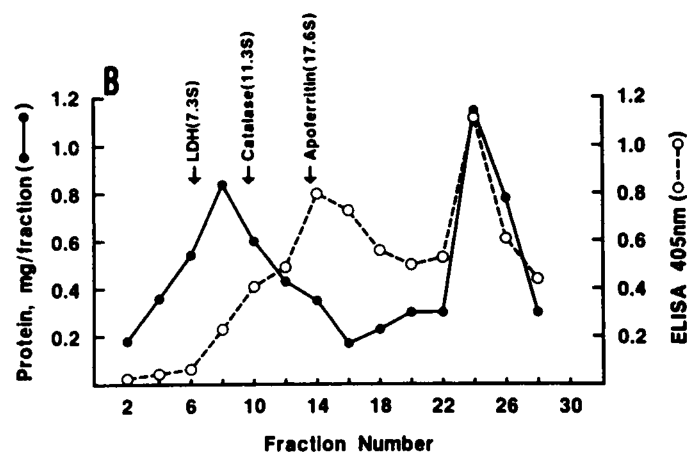


Fig. 8. Elution profile of (*A*) 0.01 M Tris/0.2% sodium deoxycholate extracts of HeLa cell nucleoli after RNase treatment and fractionated on sucrose density gradients, and (*B*) a similar HeLa cell nucleolar extract but untreated with RNase. The gradient was 5–45% sucrose; the sample was centrifuged for 18 h at 80,000 g in a SW-28 rotor (4°C) and collected in 1.2-ml fractions. The fractions were assayed for immunoreactivity by enzyme-linked immunosorbent assay using MAb FB2 (○) or assayed for protein concentration (●). Increasing fraction numbers correspond to increasing sucrose concentration. *LDH*, lactate dehydrogenase.

retinoic acid-treated and untreated HL-60 cells and from 72-h serum-deprived and serum-fed HeLa cells. This study showed that p120 was not detectable or was in trace amounts in retinoic acid and treated HL-60 cells (Fig. 7*B*) and in growth-arrested HeLa cells (Fig. 7*D*). The p120 antigen was readily detectable on immunoblots of nuclei from non-retinoic acid-treated HL-60 cells and from serum-fed HeLa cells (Fig. 7, *A* and *C*). Immunoblots prepared from whole cells (not shown) failed to show a positive staining p120 band, probably indicating the need to enrich the p120 content by preparing nuclear or nucleolar extracts.

Antigen Characterization. RNase-treated and non-RNase-treated HeLa cell nucleolar extracts were fractionated on 5–45% linear sucrose density gradients (Fig. 8, *A* and *B*). The enzyme-linked immunosorbent assay activity of individual gradient fractions indicated that p120 immunoreactivity was spread across the gradient; the highest concentration was in the high-molecular-weight region (>17S) in both RNase-treated and nontreated extracts (Fig. 8, *A* and *B*). In some experiments, slight shifts to a lower-molecular-size particle was observed for p120 in RNase-treated samples (Fig. 8*B*). To further analyze the RNase-treated samples, fractions from the sucrose gradient of RNase-treated nucleolar extracts were electrophoresed on SDS polyacrylamide gels and stained with silver (Fig. 9*A*) or gels were subjected to Western transfer and immunostained with the MAb FB2 (Fig. 9*B*). Most of the p120 immunoreactivity was observed in the higher-molecular-weight fractions (Fractions 21–28) (Fig. 9*B*) in agreement with the enzyme-linked immunosorbent assay activity (Fig. 8*A*). A M_r 120,000 peptide was observed on silver-stained gels in these fractions (Fig. 9*A*, *arrow*). Repeated experiments failed to show any significant alteration in the size of the particle containing p120 following RNase digestion.

DISCUSSION

In this study, we used an immunomasking strategy (25) for development of a MAb (FB2) to a M_r 120,000 nucleolar antigen which was detected in a broad range of tumors, including cancers of the gastrointestinal tract, genitourinary tract, blood, lymph system, brain, liver, lung, and breast (Table 1). Antigen p120 was not detected in corresponding normal tissues. However, antigen p120 was detectable in some proliferating non-malignant tissues (Table 2) such as testis and ductal regions of hypertrophied prostates. One Hodgkin's lymphoma and one breast tumor exhibited only weak nucleolar immunofluorescence with MAb FB2.

Examination of additional tumors is needed to establish whether the lack of staining or the weaker reactivity of some tumors or cells with MAb FB2 relates to patient response to treatment, type, or staging of the tumor, degree of proliferation, or technical problems in specimen processing. Quantitative studies are also needed to determine whether the relatively intense immunofluorescence of most tumors compared to the lesser intensity staining of normal proliferating cells (*i.e.*, testes) reflects a higher concentration of p120 in some cancer cells.

To test whether detection of p120 in tumor cells and proliferating normal cells is associated with cell proliferation, several model systems were used. Nucleolar antigen p120 was not detectable in 48-h serum-deprived HeLa cells. At intermediate time points of serum deprivation, a decrease in immunofluorescence intensity was observed for p120. Following serum replenishment, antigen p120 was detectable by specific nucleolar immunofluorescence within 30 min. The intensity of immunofluorescence returned to control levels by 2 h. Antigen p120

Downloaded from http://aacrjournals.org/cancerres/article-pdf/48/5/1244/2434853/cr0480051244.pdf by guest on 24 August 2022

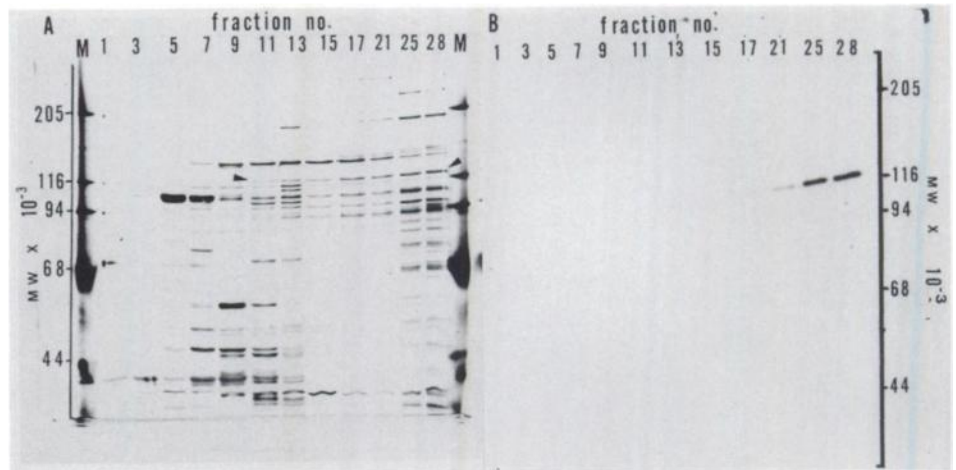


Fig. 9. *A*, silver-stained gel profile for individual fractions from the sucrose density gradient of RNase-treated 0.01 M Tris/0.2% sodium deoxycholate extracts of HeLa cell nucleoli in Fig. 8*A*. From each fraction used, 100 µl were mixed with 50 µl of 3× Laemmli buffer, electrophoresed on a 7.5% polyacrylamide gel, and stained with silver as indicated in "Materials and Methods." *B*, a corresponding immunoblot stained as in Fig. 1 with the MAb to nucleolar antigen p120. *Arrows* in *A*, band corresponding to the molecular weight of the immunoreactive band observed in Fig. 8*B*.

was detectable in 48-h PHA-stimulated lymphocytes (Fig. 4). Conversely, antigen p120 was lost upon termination of proliferation in retinoic acid-differentiated HL-60 cells. These results suggest that p120 is temporally expressed in the G_1 phase.

Nucleolar antigen p120 differs from cyclin, PCNA p145 (14, 19), and the PCNA p40 (15) by molecular size, peptide maps (not shown), and time of cell cycle expression. Antigen p120 is expressed rapidly (within 30 min) following serum replacement in growth-arrested HeLa cells. We have observed the presence of p120 in some HeLa cells as early as 15 min following refeeding; the immunofluorescence intensity reached a maximum at 2 h and persisted throughout the cell cycle. Antigen p145 persisted in growth-arrested tumor cells (14) and p40 was expressed at 4–6 h following addition of serum (15). The PCNA cyclin was reported (17) to be synthesized at the G_1/S border. The early G_1 expression of p120 is similar to that reported (24) for the nuclear oncogene c-*fos*. However, antigen p120 differs from c-*fos* as well as the nuclear oncogenes c-*myc* and c-*myb* (24) in molecular size and its specific nucleolar localization. Antigen p120 is also unlikely to be a subunit of RNA polymerase I since antibodies to this enzyme localize to nucleolar fibrillar component whereas p120 appears to be excluded from this region (data not shown).

Like antigen p145, antigen p120 is associated with a particle (>17S). However, the particle containing the p120 peptide is only slightly altered in size following RNase treatment while the p145 antigen is readily released by RNase to a smaller particle (~10S).

Proteins synthesized during G_1 may serve many functions in cell proliferation (23, 24, 29–31). In normal cells the synthesis of the control proteins may be regulated by external growth factors (22). The need for external stimuli for expression of these regulatory proteins may be eliminated or short-circuited in cancer cells.

The association of antigen p120 with proliferating cells and its specific time of expression in early G_1 suggest it plays a role in cell proliferation. This antigen may be useful as a marker for early G_1 and may have immunodiagnostic value as a marker for cell proliferation.

REFERENCES

1. Busch, H., Busch, R. K., Chan, P. K., Chatterjee, A., Freeman, J., Ross, B., Black, A., Yaneva, M., and Durban, E. Nucleolar antigens in cancer tissues. J. Tumor Marker Oncol., *1:* 69–80, 1986.
2. Busch, H., Gyorkey, F., Busch, R. K., Davis, F. M., Gyorkey, P., and Smetana, K. A nucleolar antigen found in a broad range of human malignant tumor specimens. Cancer Res., *39:* 3024–3030, 1979.
3. Busch, H., Busch, R. K., Chan, P.-K., Isenberg, W., Weigand, R., Russo, J., and Furmanski, P. Results of a "blind" study on the presence of the human tumor nucleolar antigen in breast carcinomas, benign breast tumors and normal breast tissues. Clin. Immunol. and Immunopathol., *18:* 155–167, 1981.
4. Busch, H., Busch, R. K., Chan, P. K. Kelsey, D., and Takahashi, K. Nucleolar antigens of human tumors. Methods Cancer Res., *19:* 109–178, 1982.
5. Busch, R. K., Daskal, I., Spohn, W. H., Kellermayer, M., and Busch, H. Rabbit antibodies to nucleoli of Novikoff hepatoma and normal liver of the rat. Cancer Res., *34:* 2362–2367, 1974.
6. Davis, F. M., Busch, R. K., Yeoman, L. C., and Busch, H. Differences in nucleolar antigens of rat liver and Novikoff hepatoma ascites cells. Cancer Res., *38:* 1906–1915, 1978.
7. Davis, F. M., Gyorkey, F., Busch, R. K., and Busch, H. A nucleolar antigen found in several human tumors but not in nontumor tissues. Proc. Natl. Acad. Sci. USA, *76:* 892–896, 1979.
8. Chan, P.-K., Busch, R. K., Takahashi, K., and Busch, H. Purification of human tumor nucleolar antigens to electrophoretic homogeneity. Transplant Proc., *8:* 1955–1957, 1981.
9. Chan, P.-K., Feyerbend, A., Busch, R. K., and Busch, H. Identification and partial purification of human tumor nucleolar antigen 54/6.3. Cancer Res., *40:* 3194–3201, 1980.
10. Chan, P.-K., Frakes, R. L., Busch, R. K., and Busch, H. Isolation and partial characterization of a nuclear antigen (68/6.3) from the Namalwa cell line (a Burkitt lymphoma). J. Cancer Res. Clin. Oncol., *103:* 7–16, 1982.
11. Takahashi, K., Chan, P. K., Busch, P. K., and Busch, H. Identification and purification of Namaleva Nuclear RNP Antigen 52/5.3. J. Cancer Res. Clin. Oncol., *105:* 67–75, 1983.
12. Spohn, W. H., Ahn, Y. S., Busch, R. K., and Busch, H. Electrophoretic analysis of HeLa cell and human liver nucleolar proteins and antigens. Cancer Invest., *3:* 307–320, 1985.
13. Freeman, J. W., McRorie, D. K., Busch, R. K., Gyorkey, F., Gyorkey, P., Ross, B. E., Spohn, W. H., and Busch, H. Identification and partial characterization of a nucleolar antigen (p145) found in a broad range of human cancers. Cancer Res., *46:* 3593–3598, 1986.
14. Freeman, J. W., Dowell, B. L., Ochs, R. L., Ross, B. E., and Busch, H. Effect of differentiation on the expression of nucleolar antigen p145 in HL-60 cells. Cancer Res., *47:* 586–591, 1987.
15. Chatterjee, A., Freeman, J. W., and Busch, H. Identification and partial characterization of a M_r 40,000 nucleolar antigen associated with cell proliferation. Cancer Res., *47:* 1123–1129, 1987.
16. Bravo, R., Frey, S. J., Bellatin, J., Morse, L. P., Celis, A., and Celis, J. E. Identification of a nuclear and of a cytoplasmic polypeptide whose relative proportions are sensitive to changes in the rate of cell proliferation. Exp. Cell Res., *136:* 311–315, 1981.
17. Celis, J. E., Madsen, P., Nielsen, S., and Celis, A. Nuclear patterns of cyclin (PCNA) antigen distribution subdivide S-phase in cultured cells—some application of PCNA antibodies. Leukemia Res., *10:* 237–249, 1986.
18. Chan, P.-K., Aldrich, M., Tan, E. M., and Busch, H. Detection of a 140 kD nucleolus protein with an anti-PCNA autoantibody. Cell Biol. Int. Rep., *9:* 61–68, 1985.
19. Mathews, M. B., Bernstein, R. M., Franza, B. R., and Garrels, J. L. Identity of the proliferating cell nuclear antigen and cyclin. Nature (Lond.), *309:* 374–376, 1983.
20. Smetana, K., Gyorkey, F., Chan, P.-K., Tan, E., and Busch, H. Proliferating cell nuclear antigen (PCNA) and human malignant tumor nucleolar antigens (HMTNA) in nucleoli of human hematological malignancies. Blut, *46:* 133–141, 1983.
21. Tan, E. M. Autoantibodies to nuclear antigens (ANA): their immunobiological and medicine. Adv. Immunol., *33:* 167–240, 1982.
22. Burgess, A. Growth factors and oncogenes. Immunology Today, *6:* 107–112, 1985.
23. Thompson, C. B., Challoner, P. B., Neiman, P. E., and Groudine, M. Expression of c-*myb* proto-oncogene during cellular proliferation. Nature

Downloaded from http://aacrjournals.org/cancerres/article-pdf/48/5/1244/2434853/cr0480051244.pdf by guest on 24 August 2022

(Lond.), *319:* 363–367, 1986.
24. Verrier, B., Muller, D., Bravo, R., and Muller, R. Wounding of fibroblast monolayer results in rapid induction of the c-*fos* proto-oncogene. EMBO, *5:* 913–917, 1986.
25. Freeman, J. W., Busch, R. L., Ross, B. E., and Busch, H. Masking of nontumorous antigens for development of human tumor nucleolar antibodies with improved specificity. Cancer Res., *45:* 5637–5642, 1985.
26. Takacs, B. Polyacrylamide gel electrophoresis. *In:* I. Lefkovits and B. Pernis (eds.), Immunological Methods, pp. 81–105. New York: Academic Press, 1979.
27. Towbin, H., Stahelin, T., and Gordan, J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets procedure and some applications. Proc. Natl. Acad. Sci. USA, *76:* 4350–4354, 1979.
28. Hilgers, J., Nowimsky, R. C., Geering, G., and Hardy, L. Detection of avian and mammalian oncogenic viruses by immunofluorescence. Cancer Res., *32:* 98–105, 1972.
29. Coppock, D. L., and Pardee, A. B. Regulation of thymidine kinase activity in the cell cycle by a labile protein. J. Cell. Physiol., *124:* 269–274, 1985.
30. Pardee, A. B., Dubrow, R., Harlin, J. L., and Kletzein, R. F. Animal cell cycle. Annu. Rev. Biochem., *47:* 715–750, 1978.
31. Pardee, A. B. Molecules involved in proliferation of normal and cancer cells. Ann. Assoc. Cancer Res., *27:* 433, 1986.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/48/5/1244/2434853/cr0480051244.pdf by guest on 24 August 2022