ANTIGENIC DIFFERENCES IN NUCLEAR PROTEINS OF NORMAL LIVER AND HEPATOMA*

Identification of a Nuclear Protein Present in Hepatocytes but Absent in Hepatoma Cells

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Two main types of proteins are associated with chromatin. The histones are a well characterized group of basic proteins, which seem to be involved in the general organization of the chromatin. The nonhistone proteins (NHP)[1] are a much more heterogeneous group of proteins and are believed to be involved in the regulation of gene expression (1, 2).

If gene expression is related to the composition of the NHP fraction, qualitative and quantitative changes in this composition should occur during normal differentiation, accompanying changes in growth state of cells and during carcinogenesis. Experimental evidence obtained by gel electrophoresis (3) and especially by comparison of the antigenic properties of NHP from various sources has borne out this suggestion (4).

Comparison of NHP of a given normal tissue or cultured cells and its malignant counterpart also reveals extensive antigenic differences (5–8). These early studies were carried out by using complement fixation tests with the whole NHP fraction as antigen. The individual components of NHP were not identified. More recently, Yeoman et al. (9) have isolated and characterized a nonhistone protein antigen present in rat hepatomas, but absent in the normal liver. This protein also seemed to be present in the fetal liver. In this sense it resembles the oncofetal proteins such as alphafetoprotein (10) and carcinoembryonic antigen (11). The presence of oncofetal chromatin antigens in rat hepatomas has also recently been reported by Chiu et al. (12). The identification and characterization of the various protein components of chromatin where malignant and normal tissues differ are of prime importance. If nonhistone proteins do control gene expression, such components may be highly relevant to the understanding of normal differentiation and cancer. In addition, they may provide new useful tumor markers.

We report here antigenic differences between the nuclear proteins of normal mouse liver and hepatomas and describe a nuclear protein present in normal liver, but absent in hepatomas. We also present evidence that this protein is identical to the mouse urinary protein (MUP).

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[1] *Abbreviations used in this paper:* MUP, mouse urinary protein; NHP, nonhistone protein; PCA, perchloric acid; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; SE buffer, 0.075 M NaCl, 0.025 M EDTA, 0.01 M Tris, pH 7.5.

Materials and Methods

Mice. C57L/J and C3H/HeJ mice (The Jackson Laboratory, Bar Harbor, Maine) with the transplantable hepatomas BW7756 and H4, respectively, Balb/cJ mice also from The Jackson Laboratory, and mice from two colonies of outbred mice were used. The transplantable hepatomas were propagated in male mice. To obtain spontaneous hepatomas, C3H mice were kept until more than 1 yr old. The presence of hepatoma was detected from elevated serum alphafetoprotein levels.[2]

Preparation of Nuclear Proteins. Fresh tissue was used in all preparations. Nuclei were prepared by homogenization of liver tissue in hypotonic buffer with protease inhibitors and centrifugation through 2.4 M sucrose (13, 14). The white nuclear pellet was washed and extracted by three different procedures.

TECHNIQUE NO. 1. The first technique was a modification of the procedure of Yeoman et al. (9). The nuclear pellet was resuspended in 0.075 M NaCl, 0.025 M EDTA, 0.01 M Tris, pH 7.5 (SE buffer) by using 1.0 ml of buffer for each mouse liver in the initial preparation. A sample of the nuclear pellet was sonicated to produce a whole nuclear lysate. The rest of the nuclei were washed twice in SE buffer with 10-min spins at 500 *g*. The supernates represent the first and second SE washes. The nuclei were then washed once in 0.01 M Tris, pH 7.5. This supernate is the Tris wash. The pellet was then resuspended in 3 M NaCl-7 M urea, by using 3 ml per original mouse liver. This was stirred at 4°C for 30 min then dialyzed against 10 vol of 0.15 M NaCl, 0.01 M Tris, pH 7.5 with three changes over a period of 24 h. The histone-DNA complex was centrifuged out at 10,000 *g* for 30 min and the NHP remained in the supernate. The washes were brought to 10^{-3} M with PMSF (phenylmethylsulfonyl fluoride) by using $^1/_{100}$ volume of 10^{-1} M PMSF in ethanol.

TECHNIQUE NO. 2. In the second technique, mouse liver nuclei were first washed in 0.05 M Tris, pH 7.5, 0.0002 M $MgCl_2$ to give the first Tris wash; then in 0.15 M NaCl, 0.0002 M $MgCl_2$, 0.01 M Tris, pH 7.5, to give the 0.15 M NaCl wash; then in 0.01 M Tris, pH 7.5, 0.0002 M $MgCl_2$ to give the second Tris wash. The pellet was resuspended in 2 M NaCl, 0.0002 M $MgCl_2$, 0.01 M Tris, pH 7.5, and a sample of this represented the 2 M NaCl extract. The remainder was centrifuged at 5,000 *g* for 10 min to pellet the nuclear matrix proteins (13). These were washed four more times in the 2 M NaCl buffer to give nuclear matrix. The 2 M NaCl extracted chromatin was diluted to 0.15 M NaCl, allowed to sit in the cold for 20 min, and the DNA-histone precipitate centrifuged at 10,000 *g* for 10 min. The supernate represents the nonhistone proteins.

To obtain a cytoplasmic extract one mouse liver was homogenized in 0.01 M Tris, pH 7.5, 0.0001 M $CaSO_4$, 0.001 M $MgCl_2$, 0.0001 M $NaHSO_3$, 1 μg/ml soybean trypsin inhibitor (14). The homogenate was centrifuged at 10,000 *g* for 10 min, and the supernate then centrifuged at 100,000 *g* for 1 h.

All fractions were brought to 10^{-3} M with PMSF and dialyzed overnight against several changes of distilled water.

TECHNIQUE NO. 3. A sample of the nuclei was sonicated to provide a whole nuclear lysate. The rest of the nuclei were washed twice in saline-EDTA, once in 0.01 M Tris, pH 7.5, and once in 0.035 M NaCl, 0.01 M Tris, pH 7.5. The final pellet was resuspended in distilled water and sonicated.

The protein extracts were either examined as is or lyophilized. The dry proteins were taken up in distilled water, or, if not completely soluble, in 8 M urea and 50 mM Tris. This was diluted to give a solution of 4 mg/ml in 2 M urea for immunodiffusion tests and 1:50 or more for radioimmunoassays.

Purification Procedures. Perchloric acid (PCA) extraction of normal liver and hepatoma tissue was performed as follows: the tissue was mixed with an equal volume of distilled water and homogenized in a Waring blender or a glass homogenizer. An equal vol of 1.2 M PCA was added and the precipitate was removed by centrifugation. The supernate was dialysed against water and lyophilized. Gel filtration was performed in Sephadex G-75 columns equilibrated with 0.05 M Tris-HCl, pH 7.5. The column was calibrated with proteins of known molecular weights. Ion exchange chromatography was carried out by using DE-52 cellulose (Whatman, Inc., Clifton, N. J.) equilibrated with 0.02 M sodium phosphate, pH 7.5, and eluted with a linear gradient of 0–1 M NaCl in the same buffer.

Antisera. Rabbits were immunized with single cell suspensions of normal liver cells and hepatoma cells. The suspensions were obtained from tissues cut into small pieces and incubated

[2] H. Jalanko, E. Engvall, and E. Ruoslahti. Manuscript in preparation.

with 0.1% collagenase in serum-free medium for 30 min at 37°C. The cell suspension was passed through a nylon net and about 0.2 ml of packed cells mixed with complete Freund's adjuvant was used for injection of rabbits. The rabbits received three subcutaneous injections at 2-wk intervals, after which they were bled, and then injected and bled once a month.

Immunizations with purified proteins were carried out with a similar injection schedule by using 0.2 mg of protein per injection.

Purification of Antibodies. An absorbent (15) was prepared by coupling 0.5 mg of the nuclear antigen purified from mouse liver to 250 mg of cyanogen bromide-activated Sepharose (Pharmacia, Uppsala, Sweden) according to the manufacturers instructions. Antibodies to the normal nuclear antigen were purified from an antiserum prepared against whole liver cells as follows: the antiserum (10 ml) was stirred with the insolubilized antigen for 3 h at room temperature. The particles (about 0.5 ml) were washed three times with phosphate-buffered saline and eluted four times with 1 ml of 0.1 M glycine-HCl, pH 2.6. The eluates were combined, neutralized with 1 M Tris, dialysed against distilled water, and lyophilized.

Immunoassays. Immunodiffusion plates were cast in a barbital buffer, pH 7.6. The nuclear protein samples were dissolved in 2 M urea 50 mM with Tris-HCl for testing. PCA extracts and fractions derived from them were tested dissolved in water. Since initial experiments indicated that the immunological reactivity of the antigen was not altered by treatment with urea, samples of liver and hepatoma tissue were also prepared for radioimmunoassay by extraction with urea and salt. Tissues were homogenized in water as described above. The homogenate was made 6 M with urea, 2 M with NaCl, and 10^{-4} M with PMSF. After incubation for 15 min and removal of insoluble material by centrifugation, the extract was diluted directly for testing in radioimmunoassay.

Radioimmunoassay of the nuclear antigen was performed by the double antibody method with 0.05% gelatin as diluent as described (16). Purified antigen was labeled with ^{125}I by using the chloramine-T procedure. A standard curve was obtained by using various amounts of purified antigen. The amount of antigen in the standard solution was determined by the Lowry et al. assay (17).

Immunofluorescence was performed as described (18) by using the affinity-purified antibodies as the first antibody and fluorescent anti-rabbit IgG for the visualization of the binding of antibodies. The tissues were fixed in 3.5% formaldehyde. The specificity controls included the use of normal rabbit IgG instead of the antibody and inhibition of the fluorescence with purified antigen.

Analytical Procedures. Gel electrophoresis in the presence of sodium dodecyl sulphate (SDS) was done following the procedure of Fairbanks et al. (19).

Amino acid analysis was done on samples hydrolysed with 3 N *p*-toluene sulfonic acid, 0.2% 3-(2-aminoethyl) indole at 110°C for 24 h. The hydrolysates were analysed on a Beckman 120 C amino acid analyser (Beckman Instruments, Inc., Cedar Grove, N. J.).

Electrofocusing was carried out by using an LKB Instruments, Inc., Rockville, Md. 120 ml column and ampholines in the pH range of 4–6.

Results

Identification of Nuclear Protein Differences between Liver and Hepatoma. Antisera prepared against cells from normal C57L liver and hepatoma BW7756 reacted with a number of antigens present in normal liver, hepatoma, and serum when tested in immunodiffusion. However, the anti-liver serum gave a single precipitate against nuclear protein fractions from normal liver, but did not react with similar fractions from hepatoma (Fig. 1 A). With the anti-hepatoma serum one precipitin line was produced against the hepatoma nuclear protein fraction, but the component detected seemed to be reduced in normal liver (Fig. 1 B). The antigenic component present in the nuclear protein fraction of normal liver is the subject of this report.

Purification of the Nuclear Antigen. Purification of the nuclear antigen identified in normal liver was made possible by the observation that it is soluble

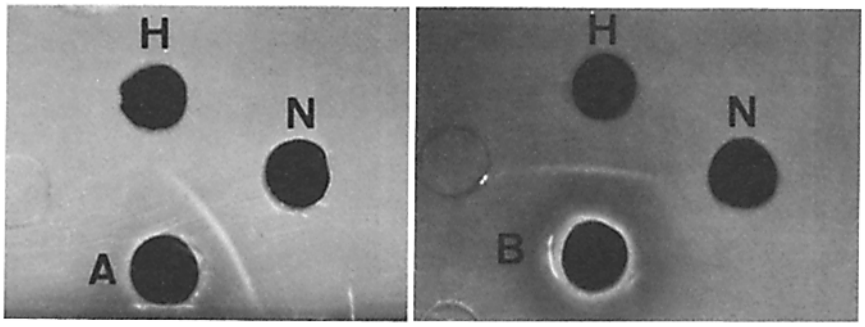

FIG. 1. Immunodiffusion analysis of nuclear protein fractions from normal liver (N) and hepatoma tissue (H). A. Antiserum to normal liver cells. B. Antiserum to hepatoma cells.

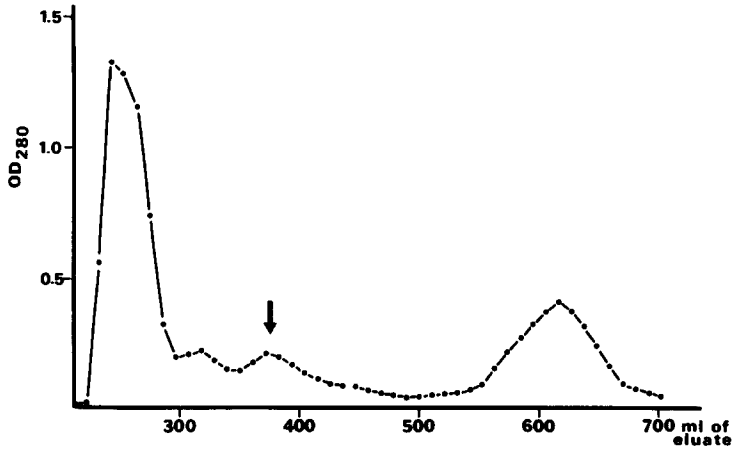

FIG. 2. Gel filtration of PCA extract of normal mouse liver on Sephadex G-75. The marked peak contained the nuclear antigen.

in 0.6 M PCA. This enabled us to start the purification by PCA extraction of whole liver. About 200 mg of extract was obtained from 50 mouse livers. Immunodiffusion indicated that nuclear antigen was a major component of such an extract. Subsequent quantitation with radioimmunoassay has shown that about 10% of the PCA extract consists of this antigen. The same component was found in several liver preparations from three different mouse strains and two colonies of out-bred mice.

In gel filtration of the PCA extract on Sephadex G-75, the antigenic activity coincided with a major optical density peak (Fig. 2). Comparison of its elution position with standard proteins indicated a mol wt of about 25,000 for the antigen. SDS-polyacrylamide gel electrophoresis of the protein in this peak revealed a major band with an approximate mol wt of about 20,000 and a few minor bands. Purification of the antigen to homogeneity was achieved by chromatography on DEAE cellulose. In ion exchange chromatography it eluted as a sharp peak at NaCl concentration of about 0.4 M (Fig. 3). Electrofocusing gave an optical density peak with coinciding immunoreactivity at pH 4.50. A minor fraction of immunoreactivity was found between pH 4.75 and 5.50 (not shown).

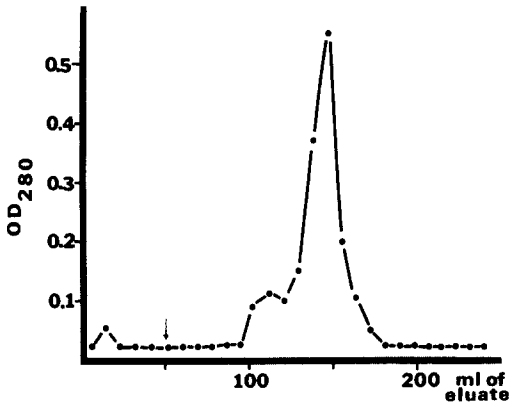

FIG. 3. Ion exchange chromatography on DEAE cellulose. A 15-ml column was equilibriated with 0.02 M phosphate buffer at pH 7.5. A 20-mg sample of partially purified nuclear antigen from gel filtration (Fig. 2) was applied, and the column was eluted with the starting buffer (50 ml) and a 200 ml 0–1 M NaCl linear gradient (arrow) in the same buffer. The antigen elutes at a NaCl concentration of about 0.4 M.

Antigen obtained from the ion exchange chromatography peak gave a single band on SDS gels (Fig. 4). Comparison of its mobility with standard proteins indicated a mol wt of 21,000. Amino acid analysis revealed an absence of proline and abundance of the acidic amino acids (Table I). The ratio of the acidic (Glx + Asx) to basic amino acids was 1.95. No amino sugars were detected. The lack of carbohydrate was confirmed by a carbohydrate analysis, which established the carbohydrate content of the antigen as less than 0.5%. Absorbance of an 0.1% solution at 278 nm with 1 cm light path was 0.600.

Subcellular Distribution of the Nuclear Antigen. The antigen was quantitated in the nuclear washes and protein fractions by using a radioimmunoassay capable of measuring 1 ng/ml of this protein. These assays of the various nuclear subfractions produced by technique nos. 1–3 indicate that it is a nuclear protein loosely bound to chromatin (Tables II–IV). In technique no. 1 all of the contaminating cytoplasmic proteins were washed out of nuclei in the first and second SE wash (14), and a subsequent Tris wash showed the highest specific activity. Most of the antigen was extracted in the various nuclear washes, and the specific activity was low in the NHP supernate, which represents the proteins more tightly bound to the chromatin. There was essentially no antigen present in the DNA-histone pellet.

In technique no. 2 the cytoplasm was assayed and found to have a relatively low specific activity compared to the 0.15 M NaCl nuclear wash. The specific activity was intermediate for the first Tris wash of the nuclei which would remove most contaminating cytoplasmic proteins (14). The specific activity of the 0.15 M NaCl wash was 14-fold greater than that of the cytoplasm and at least 8-fold greater than the 2 M NaCl extracted chromatin, the nuclear matrix, the DNA-histone, and the NHP.

Technique no. 3 confirmed the results with technique no. 1. Here again, the highest specific activity was obtained with the Tris wash long after most of the nuclear sap protein has been washed out of the nucleus (14).

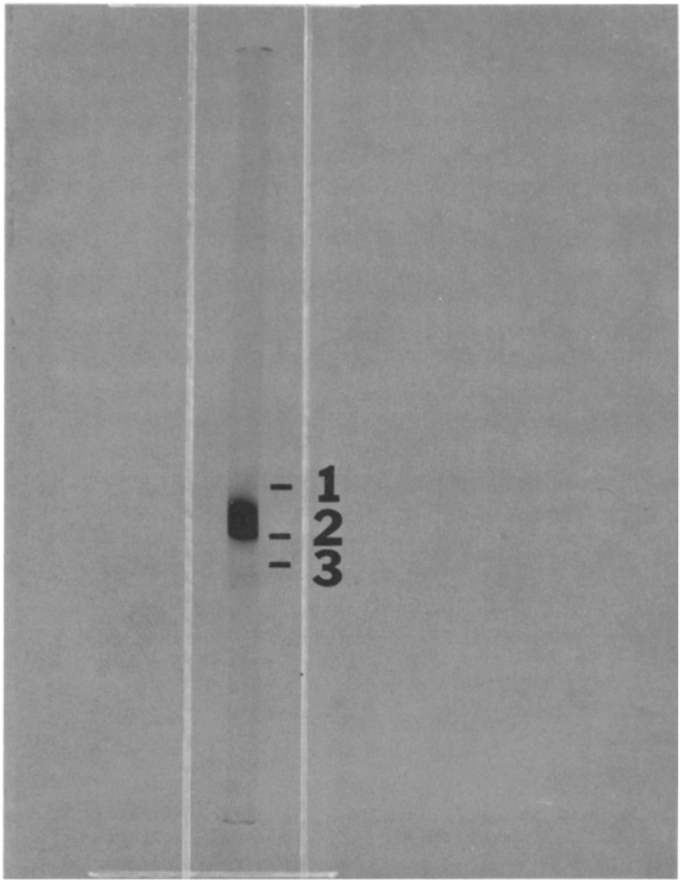

FIG. 4. Polyacrylamide gel electrophoresis in the presence of SDS. The gel sample contained 50 μg of purified nuclear antigen. The positions of molecular weight standards run on companion gels are shown. A gel with high load of protein is shown to allow evaluation of the degree of purity. The molecular weight was evaluated from gels where lower loading gave a narrower band. 1. Carbonic anhydrase (mol wt 32,000). 2. β-lactoglobulin (mol wt 18,000). 3. Lysozyme (mol wt 14,500).

Similar results were obtained on repeated experiments. No reactions were obtained when the nuclear washes and the NHP fraction were tested in immunodiffusion against anti-normal mouse serum and anti-mouse albumin.

In immunofluorescence of liver tissue with purified antibodies the antigen was found to localize mainly in the hepatocyte nuclei (Fig. 5). A granular distribution was observed suggesting that it may be bound to specific structures within the nuclei.

Tissue Distribution of the Nuclear Antigen. Tissue distribution of the antigen was studied by using radioimmunoassay of PCA and urea extracts of various mouse tissues (Table V). Of the tissues tested, the highest concentration was found in the liver. Lower levels were found in other organs. Hepatomas showed a much lower concentration of the antigen than liver tissue. Only trace amounts were found in PCA extracts of two transplantable and one spontaneous

TABLE I
Amino Acid Composition of Purified Nuclear Antigen

Amino acid	Moles/mole of protein (mol wt 21,000)*
Tyr	3.9
Phe	9.8
Trp	0.5
Lys	13.3
His	4.9
Arg	8.3
Asp	28.5
Thr	9.9
Ser	14.0
Glu	30.2
Pro	0
Gly	12.3
Ala	12.8
½ Cys	1.8
Val	7.3
Met	2.6
Ile	8.8
Leu	17.0
Amino sugars	0

* Mean of two determinations.

TABLE II
Nuclear Antigen Content in Normal Mouse Liver Fractionated by Technique No. 1

Fraction	
	ng antigen/mg protein
Nuclei	60
First SE wash	250
Second SE wash	130
Tris wash	470
NHP supernate	30
DNA-histone pellet	<3

TABLE III
Nuclear Antigen Content in Normal Mouse Liver Fractionated by Technique No. 2

Fraction	
	ng antigen/mg protein
Cytoplasm	270
First Tris wash	700
0.15 M NaCl wash	3,900
Second Tris wash	1,600
2 M NaCl extract	390
Nuclear matrix	<300
DNA-histone	<300
Nonhistone protein	460

TABLE IV
Nuclear Antigen Content in Normal Mouse Liver Fractionated by Technique No. 3

Fraction	*ng antigen/mg protein*
Whole nuclei	530
First SE wash	830
Second SE wash	750
First Tris wash	1,220
0.035 M NaCl wash	910
Washed nuclear pellet	400

hepatoma. Extraction of the C3H transplantable hepatoma with 6 M urea and 2 M NaCl revealed 2% of the amount of antigen found in similar extracts of liver tissue from male C3H mice (Table VI). Similar extraction of a spontaneous hepatoma of a C3H mouse revealed antigen concentrations of 30 and 1,730 μg/mg of protein in the tumor and the rest of the liver of this mouse, respectively. Hepatoma nuclear protein preparations similar to the ones shown in Fig. 1 (technique no. 1) did not have antigen detectable by radioimmunoassay. This excluded the presence of more than 1% of the level present in similar preparations from normal liver. The absence of significant amounts of the nuclear antigen in hepatomas was further evidenced by the lack of antibodies against it in the antiserum prepared aginst single cell suspensions of hepatoma BW 7756. While the antiserum prepared against liver cells bound significant radioactivity from the ^{125}I-labeled antigen up to a dilution 1:10^6, the anti-hepatoma serum showed no binding diluted 1:100, the highest concentration tested. Immunofluorescence also suggested the presence of decreased amounts of the antigen in a spontaneous hepatoma (Fig. 5).

Nuclear Antigen in Blood and Urine. Similarity to MUP. The nuclear antigen was found to be present in the blood and urine (Table VII). The levels found in male mice were generally higher than those of females, but the large variation present resulted in overlapping ranges of blood concentrations for males and females.

The high urinary concentrations (up to 13 mg/ml) prompted us to study the relationship of the nuclear antigen to the mouse urinary protein, MUP (20). MUP constitutes the main part of protein in normal mouse urine and is known to originate from the liver (21). Its mol wt is close to 20,000 (22, 23), similar to that of the nuclear antigen. In keeping with earlier results (23), fractionation of mouse urine on Sephadex G-75 resulted in a distinct peak at mol wt of about 25,000. The elution volume of this peak was the same as that of the nuclear antigen. The protein in this peak reacted identically with the liver nuclear antigen when tested in immunodiffusion (Fig. 6).

Discussion

The results of this study confirm that immunochemically detectable differences exist between the nuclear proteins of normal and malignant tissue (6–9). It was rather surprising that the antisera we prepared against whole cells from

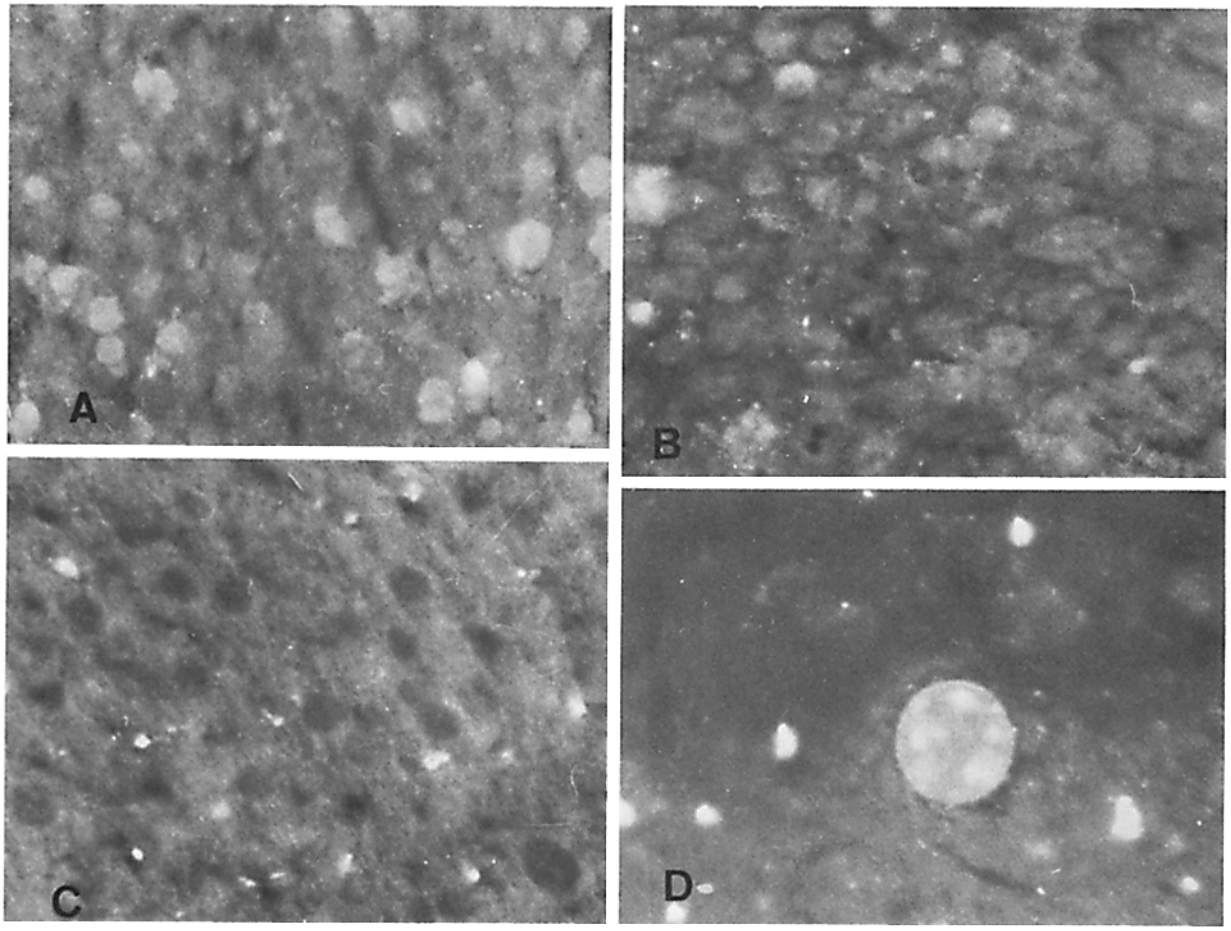

FIG. 5. Localization of nuclear antigen in sections of normal mouse liver and a spontaneous C3H hepatoma by immunofluorescence. The antigen is mainly found in the nuclei with some staining of the cytoplasm. Weaker staining of the hepatoma nuclei is evident. (A) Normal liver stained with anti-nuclear antigen. (B) Spontaneous hepatoma stained with anti-nuclear antigen. (C) Normal liver stained with normal rabbit serum as a control. (D) Higher magnification of a single nucleus from normal liver stained with anti-nuclear antigen. The controls also included neutralization of the antibody with purified antigen. This abolished the fluorescence.

normal liver reacted only with a single component in the liver nuclear proteins. This antigen was not detectable in hepatoma nuclear protein preparations. The antigen(s) detected in the hepatoma nuclear proteins will be studied further, but present results indicate that the antiserum prepared against whole hepatoma cells detected a single component. This antigen was detectable in nuclear proteins from two hepatomas, one of which was the source of the immunogen, but seemed to be present in lower amounts in the nuclear protein fractions from normal liver.

Nuclear protein preparations are known to contain up to 500 different proteins detectable by two-dimensional electrophoresis. It may be that most of these

TABLE V
Nuclear Antigen Content in PCA Extracts of Various Normal Tissues and Hepatomas

Sample	*μg antigen/mg protein*
Mouse liver 1	109
" " 2	119
" BW7756 hepatoma (C57L/J)	0.4
" H4 hepatoma (C3H/HeJ)	0.05
" Spontaneous hepatoma (C3H/HeJ)	<0.1
" Lung 1	2.0
" " 2	6.0
" Spleen	0.8
" Stomach	4.8

TABLE VI
Nuclear Antigen Concentration of Urea-NaCl Extracts of Normal Liver and Hepatomas

Tissue	*ng antigen/mg protein*
Normal Liver male C3H 1	1,890
" " 2	3,890
" " 3	2,440
" " 4*	1,730
" " female 1	730
2	410
3	1,000
Transplantable C3H hepatoma H4	80
Spontaneous C3H hepatoma*	30

* Liver and tumor from the same mouse.

TABLE VII
Nuclear Antigen Concentration in Mouse Serum and Urine

Sample	Strain and sex	Antigen concentration	
		Mean	Range
		μg/ml	
Serum	C3H/HeJ male	24.4	2.4–58.0
"	C3H/HeJ female	9.7	3.5–24.0
"	C57L/J male	14.0	1.8–38.0
Urine	C3H/HeJ male	8,100	3,500–13,100
"	C3H/HeJ female	1,600	300–2,550
"	C57L/J male	6,900	5,900–7,900

proteins are present in concentrations too low to elicit an antibody response or to be detectable by immunodiffusion. It is also possible that the proteins we have detected represent a particularly immunogenic subclass of nuclear proteins. Finally, it is possible that these proteins are unusual in that they are not only present in high concentrations in the nucleus but are also present in significant

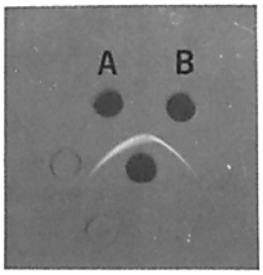

FIG. 6. Immunodiffusion analysis of the nuclear antigen purified from liver 500 μg/ml (A) and MUP complex isolated from mouse urine by gel filtration (23) 250 μg/ml (B). Tested against antiserum to purified nuclear antigen.

concentrations in the cytoplasm. Thus they have a higher overall concentration in whole cells than proteins present only in the nucleus. As could be expected, our antisera reacted with a number of other components present in cytoplasmic proteins or serum. Whatever the reason for their apparent specificity of our antisera when reacted against nuclear proteins, the results demonstrate the usefulness of immunochemical techniques in the identification of individual nuclear proteins.

The identification, isolation, and characterization of a nuclear NHP from normal liver reported here provides new information about a protein different from any of the few nuclear proteins previously isolated. Its amino acid composition and/or molecular weight differ from those of the HMG proteins (24–26), A24 (27), and from those of the J_2 protein recently identified in the mouse liver nuclei.[3] However, it is apparent that the nuclear protein we describe here is closely related, if not identical, to mouse urinary protein, MUP (20).

Mice, unlike humans, excrete a large amount of protein consisting mainly of MUP in the urine (20–23). It is known that MUP is also present in serum, and that it originates in the liver (21). Genetic variation occurring in this protein has been used as a marker to study genetically mosaic liver (28). The expression of MUP is partly sex-dependent, with higher expression in males. Testosterone treatment of castrated females brings their MUP expression to male level (20).

We found the nuclear protein antigen to share several of these properties with MUP. Concentrations up to 13 μg/ml were present in the urine, and the antigen made up 50–90% of the nondialysable material in urine. The serum levels of MUP have been reported to be 50–150 and 10–20 μg/ml in male and female mice, respectively (29). We found similar serum concentrations for the nuclear protein, including the sex difference observed for MUP. Our results indicate a larger variation in these levels than those previously reported for MUP (21). Like MUP, our protein appeared to be present predominantly in the liver. While the smaller amounts of nuclear antigen we found in various other organs could originate from the blood and tissue fluid present in these organs, a low level expression of the nuclear antigen in organs other than the liver cannot be excluded. The mol wt of MUP has been reported to be 17,500 (23) and 20,000 (22). These values are in an agreement with our estimate of 21,000 for the nuclear

[3] B. J. Conner and D. E. Comings. Nuclear proteins IV. Studies of histone-binding proteins from mouse liver by affinity chromatography. Manuscript submitted for publication.

antigen. These similarities in the expression and molecular properies of MUP and the nuclear antigen we describe clearly show that the two proteins are related if not identical. The function of MUP is not known. It has been suggested that it could be involved in the transportation of testosterone or its metabolites to the urine. Measurement of binding of the hormone by this protein, however, revealed a very low association constant (30). To our knowledge the nuclear localization of MUP has not been reported before.

Apart from being nuclear protein, our antigen shows the interesting characteristic of being absent from malignant hepatocytes. Our results show that two transplantable hepatomas and three different spontaneous hepatomas have less than 2% of the amount present in normal liver tissue of mice corresponding strains. The amount in hepatomas is comparable to what we found in organs other than the liver and at least partly originates from the blood present in the tumor tissues. This suggests a virtual absence of the antigen in hepatomas. The transplantable hepatomas used in this study may have accumulated a number of biochemical changes unrelated to the expression of malignancy during the many in vivo passages they have been through. It is therefore significant that spontaneous hepatomas, which are more likely to represent a malignancy-associated deviation from the normal phenotype, also showed a decreased amount of the antigen.

The loss of normal components is a well established phenomenon for various tumors, including hepatomas (31). The nuclear localization and hormone dependence of MUP suggest that it could have a regulatory function in maintenance of the normal phenotype of liver cells.

Summary

A nuclear antigen was detected in the mouse liver nonhistone protein fraction by using antibodies to whole liver cells. The antigen was purified to homogeneity from perchloric acid extracts of liver tissue. It gave a single band corresponding to mol wt 21,000 in sodium dodecyl sulfate gel electrophoresis. Amino acid and carbohydrate analysis showed predominance of the acidic amino acids, lack of proline, and absence of carbohydrate.

Immunofluorescence staining of liver sections confirmed the nuclear localization of the antigen. Its tissue distribution was studied by using radioimmunoassay. Of the various tissues extracted for analysis, the liver contained the highest amounts of the antigen, about 1 μg/mg of solubilized liver protein. Other tissues examined showed 2–4% of the amount of antigen present in the liver. Two transplantable hepatomas in C3H/HeJ and C57L/J mice, respectively, and three spontaneous C3H hepatomas showed greatly decreased levels of the antigen compared to normal liver. The amount of antigen in hepatomas varied from nondetectable to 2% of the amount of antigen found in the livers of the mice. The antigen was also found in the blood.

The antigen was found in high concentrations (up to 13 mg/ml) in the urine of normal mice. This suggests identity with the previously known mouse urinary protein (MUP). In addition to the extremely high urinary output, the properties found to be shared by MUP and the nuclear antigen included similar serum concentrations (2–60 μg/ml), a sex difference with lower values in females, same

molecular size as determined by gel filtration, and immunological identity. The nuclear localization of MUP and its disappearance from hepatomas suggest that it may have an important regulatory function.

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