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Characterization of the cDNA Encoding Human Nucleophosmin and Studies of Its Role in Normal and Abnormal Growth†

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ABSTRACT: A cDNA encoding human nucleophosmin (protein B23) was obtained by screening a human placental cDNA library in λgtll first with monoclonal antibody to rat nucleophosmin and then with confirmed partial cDNA of human nucleophosmin as probes. The cDNA had 1311 bp with a coding sequence encoding a protein of 294 amino acids. The identity of the cDNA was confirmed by the presence of encoded amino acid sequences identical with those determined by sequencing pure rat nucleophosmin (a total of 138 amino acids). The most striking feature of the sequence is an acidic cluster located in the middle of the molecule. The cluster consists of 26 Asp/Glu and 1 Phe and Ala. Comparison of human nucleophosmin and *Xenopus* nucleolar protein NO38 shows 64.3% sequence identity. The N-terminal 130 amino acids of human nucleophosmin also bear 50% identity with that of *Xenopus* nucleoplasmin. Northern blot analysis of rat liver total RNA with a partial nucleophosmin cDNA as probe demonstrated a homogeneous mRNA band of about 1.6 kb. Similar observations were made in hypertrophic rat liver and Novikoff hepatoma. However, the quantity of nucleophosmin mRNA is 50- and 5-fold higher in Novikoff hepatoma and hypertrophic rat liver, respectively, when compared with normal rat liver. Dot blot analysis also showed a nucleophosmin mRNA ratio of 64:5:1 in the three types of rat liver. When the protein levels were compared with Western blot immunoassays, Novikoff hepatoma showed 20 times more nucleophosmin, while only about 5 times more nucleophosmin was observed in hypertrophic rat liver than in unstimulated normal liver.

Nucleophosmin (formerly known as protein B23, 38-kD, numatrin, and NO38) is a nucleolar phosphoprotein which is more abundant in tumor cells than in normal resting cells (Olson et al., 1974; Busch et al., 1984; Fields et al., 1986; Feuerstein & Mond, 1987; Schmidt-Zachmann et al., 1987; Feuerstein et al., 1988). Stimulation of normal cells to grow is accompanied by an increase in nucleophosmin protein level as demonstrated in mitogen activation of B lymphocytes (Feuerstein & Mond, 1987; Feuerstein et al., 1988). Even though the function of nucleophosmin has not been defined, there is ample evidence suggesting that it is involved in the assembly of ribosomal proteins into ribosomes (Prestayko et al., 1974; Yung et al., 1985a; Schmidt-Zachmann et al., 1987). Electron microscopic study indicated that nucleophosmin is concentrated in the granular region of the nucleolus (Spector et al., 1984) where ribosome assembly occurs. This protein forms hexamers (Yung & Chan, 1987) and is associated with 60S and 80S preribosomal particles (RNPs) in the nucleolus (Prestayko et al., 1974; Yung et al., 1985a). When cells are treated with cytotoxic agents including actinomycin D, doxorubicin, toyocamycin, luzopeptins, and mitomycin C, which affect the morphology and function of nucleoli, nucleophosmin translocates from the nucleolus to the nucleoplasm (Yung et al., 1985a,b, 1986; Chan et al., 1987; Chan, P. K., et al., 1988), suggesting that these cytotoxic agents either alter or destroy the binding site of nucleophosmin in the nucleolus.

The amino acid sequence of the phosphorylation site of nucleophosmin, S(p)EDEDEED, has been determined (Chan et al., 1986a). It is identical with that of several other phosphoproteins, including phosphoprotein C23 and the R-II subunit of cAMP protein kinase. It has been suggested that this sequence might represent the common N-II kinase recognition site (Chan et al., 1986a). A specific antigenic peptide with a lysine-rich domain has been identified at the carboxyl terminal of the molecule (Chan et al., 1986b). In order to study the structure/function relationship of nucleophosmin and understand the organization of the different domains, such as the phosphorylation site and the protein and RNA binding sites, attempts were made to obtain the complete amino acid sequence of nucleophosmin. This paper reports the determination of the amino acid sequence of peptides purified from rat nucleophosmin and the nucleotide sequence of the cDNA of human nucleophosmin. Both antibody to and cDNA of nucleophosmin were used as probes to study the change in nucleophosmin during stimulated growth and tumorigenesis in an animal model.

Materials and Methods

Chemicals. Thioacetamide was purchased from Fisher Chemical Co. (Houston, TX). All restriction enzymes and DNA modification enzymes were supplied by either Bethesda Research Laboratories (Gaithersburg, MD) or Pharmacia (Piscataway, NJ). RNasin ribonuclease inhibitor (40 units/μL) was purchased from Promega Biotec (Madison, WI). All other chemicals used were either of reagent grade or of molecular biology grade. Radiochemicals were purchased from

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Amersham Corp. (Arlington Heights, IL).

Animals. Novikoff hepatoma ascites cells were implanted intraperitoneally into adult male albino Holtzman rats (200 g) 6 days before ascites cell collection (Busch et al., 1984). To obtain thioacetamide-stimulated liver, male albino Holtzman rats (200 g) were injected intraperitoneally with thioacetamide in 0.15 M NaCl (500 μg of thioacetamide/kg body weight) 24 h before sacrificing (Anderson et al., 1977).

Nucleophosmin Isolation, Peptide Sequencing, and Amino Acid Analysis. Novikoff hepatoma ascites cell nucleoli were isolated by the NP-40 method (Chan et al., 1986a). Nucleophosmin was extracted from purified nucleoli with 4 M urea/3 M LiCl and purified by DEAE-cellulose chromatography (Chan et al., 1985). The method for purification of peptides was essentially the same as described previously (Chan et al., 1986a,b). Peptides designated as V8-34 and Tp were purified by HPLC chromatography. The amino acid sequences of these peptides were determined by the method of Edman and Begg (1967) in a Beckman 890C liquid-phase sequenator. About 3–5 nmol of peptide was applied into the liquid sequenator. The resulting PTC derivatives from the liquid-phase sequenator were converted to PTH-amino acids and analyzed by HPLC in a reverse-phase CN column (Hunkapillar & Hood, 1983). For amino acid analysis, peptides were hydrolyzed in 5.7 N HCl at 110 °C for 22 h, and the amino acid compositions were determined by an ion-exchanger amino acid analyzer (Beckman 121 MB).

Monoclonal Antibody Preparation. Monoclonal antibodies were purified by affinity chromatography. The purified antibody was labeled with ^{125}I in the presence of iodogen (1,3,4,6-tetrachlorodiphenylglycoluril, Sigma) and 1 M Tris-HCl, pH 8.0 (Chan, W. Y., et al., 1988). The specific activity of the protein labeled by this method was 10^7 cpm/μg.

Immunological Screening and Nucleotide Screening of Human Placental cDNA Library. Initially, a human term placental cDNA library in λgtll, kindly provided by Dr. Brian Knoll (Department of Pathology, University of Texas Medical School at Houston, Houston, TX), was screened with labeled anti-rat nucleophosmin antibody using the method of Young and Davis (1983) with modifications (Chan, W. Y., et al., 1988). Three strongly positive clones were picked from about 2×10^6 recombinant phages plated out. These clones were proven to be partial cDNAs encoding human nucleophosmin (Chan et al., 1986b). Subsequently, the cDNA insert of one of these clones was labeled and used as a probe to screen another human placental cDNA library, purchased from Clontech Laboratories (Palo Alto, CA), with a procedure described previously (Chan, W. Y., et al., 1988).

DNA Sequence Analysis. Phage DNA was prepared from positive clones by using a plate lysis method (Maniatis et al., 1982), followed by banding on a cesium chloride step gradient as described by Degen et al. (1983). Alternatively, a small quantity of pure phage DNA was prepared by using the method of Benson and Taylor (1984). The nucleotide sequence of cDNA was determined by the dideoxy chain termination method (Johnston-Dow et al., 1987). M13 universal sequencing primer (Pharmacia) and also synthetic oligonucleotide primers were used to prime the sequencing reaction. cDNAs were also cloned into Riboprobe pGEM plasmids (Promega Biotech) and sequenced by dideoxy chain termination methods using the USB Sequenase protocol (Tabor & Richardson, 1987). Primers for SP6 and T7 RNA polymerase promoters and synthetic primers (U9.P1, U9.P2, U9.P3, U9.P4, 41A.P1, and G11.P, for sequencing the regions not covered by the SP6 and T7) were used. Both strands of cDNA were sequenced with 50–300 nucleotide overlaps. All sequences were determined 3 or more times and from both strands.

Preparation of Hybridization Probes of Nucleophosmin. The cDNA insert of a human nucleophosmin clone, hPB2, coding for the 82 amino acids at the carboxyl terminus of nucleophosmin and the 3′-nontranslting region (Chan et al., 1986), was used for rescreening the placental library as well as for RNA dot blot analysis. The cDNA insert was labeled with [^{32}P]dATP by nick translation (Rixon et al., 1983). Two rDNA genomic clones containing the 18S and 28S rRNA coding sequences were generous gifts from Dr. L. Rothblum, Department of Pharmacology, Baylor, College of Medicine.

Isolation of Cellular RNA. Total cellular RNA from Novikoff hepatoma cells or rat liver were isolated by using the method described by Rosen and Monahan (1982).

Dot Blot Analysis. The dot blot hybridization method is a modification of White and Bancroft (1982). One milligram of RNA was dissolved in 50 μL of 10 mM Tris-HCl (pH 7.0)/1 mM EDTA/40 units of RNasin; 30 μL of 20 × SSC and 20 μL of 37% (w/w) formaldehyde were added to the mixture and subsequently incubated at 60 °C for 15 min. The RNA samples were serially diluted (4-fold) with 15 × SSC and blotted on a nitrocellulose membrane. Hybridization was performed in 50% formamide at 42 °C for 24 h with the denatured probe. The membrane was washed (Dobner et al., 1981) and exposed to X-ray film at −80 °C.

Western Blot Analysis. Novikoff hepatoma or rat liver nuclei were isolated by using either the NP-40 detergent or the 2.2 M sucrose/Mg^{2+} method (Davis et al., 1981). The nuclei were dissolved in Laemmli SDS sample buffer (Laemmli, 1970) with 5 M urea and heated at 100 °C for 3 min before being loaded onto a 10% SDS–polyacrylamide gel. Aliquots of nuclear proteins containing equal amounts of DNA (70 μg) from each type of tissue were used. The Western blot was performed as previously described (Chan et al., 1985).

Results

Identification and Purification of Human cDNA Clones. Initial screening of the human placental expression library with labeled anti-rat nucleophosmin antibody yielded three clones, all of which contained an approximately 600 bp nucleophosmin partial cDNA (Chan et al., 1986b). Using 1 of these nucleophosmin partial cDNAs (hPB2) as probe to screen another placental cDNA library gave 16 more positive clones with insert sizes varying from 505 to 1400 bp. The cDNA inserts of all these clones cross-hybridized strongly under high stringency in Southern blot analysis, suggesting that they are highly homologous, if not identical (results not shown).

DNA Sequence Analysis. The nucleotide sequence of the cDNA insert of the longest clone (hPB6) was determined. Figure 1 shows the sequencing strategy and partial restriction enzyme map. Figure 2 shows the nucleotide and the predicted amino acid sequence of hPB6.

The cDNA insert of clone hPB6 contained 1311 bp with an in-phase stop codon TAA at nucleotide 976. Even though no in-phase stop codon was found in the 5′-nontranslated portion of the cDNA, a consensus sequence (GCCGCCACC) for the initiation of translation (Kozak, 1987) was identified at nucleotides 83–91. We therefore assigned the ATG at nucleotide positions 94–96 as the initiation methionine codon. The cDNA thus contained a 5′-noncoding sequence of 93 bp, a coding sequence of 882 bp, a 3′-noncoding sequence of 321 bp, and a 15 bp poly(A) tail. Two potential polyadenylation signal sequences (AATAAA) were found at nucleotide positions 1271–1276 and 1289–1294. The most likely poly-

Table I: Amino Acid Sequences of Nucleophosmin

peptide[a]	sequence
(1) antigenic peptide	227-SFKKQEKTPKTPKGPSSVEDIKAKMQASIEKGGSLPKVEAKFINYVKNCFRMTDQEAIQDLWQWRKSL-COOH[b]
(2) phosphorylation site	115-HLVAVEEDAES(p)EDEDEEDVK[c]
(3) tryptic peptide T1B	33-VDNDENEHQLSLR[d]
(4) V8-34	69-GSPIKVTLATLKMSVQPTVSLGGFE
(5) tryptic peptide Tp	25-ADKDYHFK
(6) polyacidic stretch	161-DEDDDDDDEEDDDEDDDDDDFDDEEAEE
(7) potential metal binding site	104-CGSGPVHISGQH
(8) karyophilic sequences	152-PQKKVK, 191-PVKKSIR

[a] Peptides 1–5 are peptide sequences from rat nucleophosmin. Peptides 6–8 are derived from human nucleophosmin cDNA. [b] Chan et al. (1986b). [c] Chan et al. (1986a). [d] Jones (1979).

Table II: Amino Acid Composition of Human Nucleophosmin[a]

amino acid	no.	%	%[b]	amino acid	no.	%	%[b]
Ala	19	6.5	5.7	Leu	19	6.5	7.1
Arg	8	2.7	3.1	Lys	33	11.2	11.1
Asn	9	3.1		Met	8	2.7	2.6
Asp	34	11.6	15.0	Phe	7	2.4	2.6
Cys	3	1.0		Pro	17	5.8	5.8
Gln	13	4.4		Ser	26	8.8	6.8
Glu	31	10.5	15.2	Thr	10	3.4	3.8
Gly	18	6.1	7.4	Trp	2	0.7	
His	5	1.7	2.4	Tyr	4	1.4	1.3
Ile	10	3.4	3.2	Val	18	6.1	7.2

acidic (Asp + Glu)	65 (22.1)
basic (Arg + Lys)	41 (13.9)
aromatic (Phe + Trp + Tyr)	13 (4.4)
hydrophobic (aromatic + Ile + Leu + Met + Val)	68 (23.1)
molecular weight	32578

[a] The sequence contains 294 amino acids. [b] Experimental data from Jones (1979).

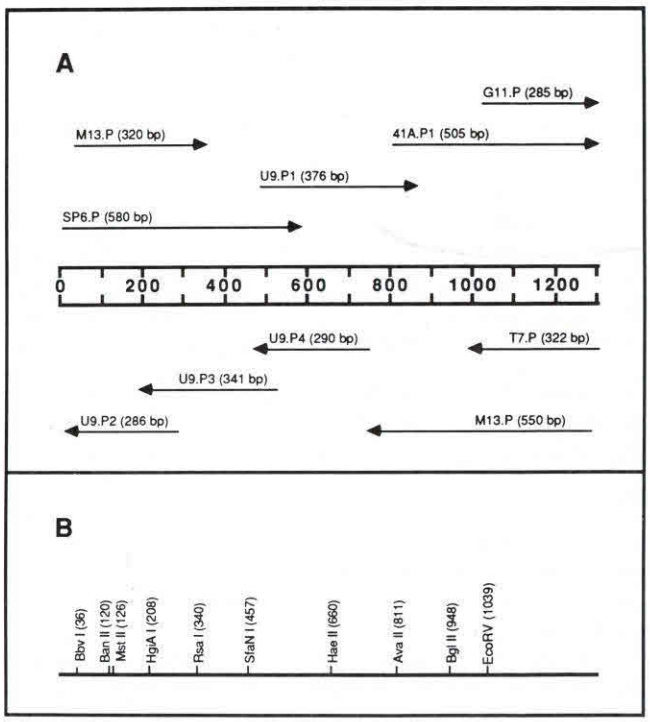

FIGURE 1: Sequencing strategy and partial restriction map of human nucleophosmin cDNA (hPB6).

adenylation signal is the former one, which is 26 bp upstream from the poly(A) tail (Proudfoot & Brownlee, 1976).

Amino Acid Sequence Analysis. Table I shows the amino acid sequences of five peptides from rat nucleophosmin (1–5 of Table I). The amino acid sequences of the antigenic peptide,

```
GGGCGGGATTCCGTCCTGCGCGGTTGTTCTCTGGAGCAGCGTTCTTTTATCTCCGTCCGC   60
CTTCTCTCCTACCTAAGTGCGTGCCGCCACCCGATGGAAGATTCGATGGACATGGACATG  120
                              MetGluAspSerMetAspMetAspMet        9

AGCCCCCTGAGGCCCCAGAACTATCTTTTCGGTTGTGAACTAAAGGCCGACAAAGATTAT  180
SerProLeuArgProGlnAsnTyrLeuPheGlyCysGluLeuLysAlaAspLysAspTyr   29
                                        -------Tp------
CACTTTAAGGTGGATAATGATGAAAATGAGCACCAGTTATCTTTAAGAACGGTCAGTTTA  240
HisPheLysValAspAsnAspGluAsnGluHisGlnLeuSerLeuArgThrValSerLeu   49
--------  --------------T1B---------------------

GGGGCTGGTGCAAAGGATGAGTTGCACATTGTTGAAGCAGAGGCAATGAATTACGAAGGC  300
GlyAlaGlyAlaLysAspGluLeuHisIleValGluAlaGluAlaMetAsnTyrGluGly   69
                                                  ---

AGTCCAATTAAAGTAACACTGGCAACTTTGAAAATGTCTGTACAGCCAACGGTTTCCCTT  360
SerProIleLysValThrLeuAlaThrLeuLysMetSerValGlnProThrValSerLeu   89
-------------------------V8-34---------------------------

GGGGGCTTTGAAATAACACCACCAGTGGTCTTAAGGTTGAAGTGTGGTTCAGGGCCAGTG  420
GlyGlyPheGluIleThrProProValValLeuArgLeuLysCysGlySerGlyProVal  109
-----------                                 * * * * * *

CATATTAGTGGACAGCACTTAGTAGCTGTGGAGGAAGATGCAGAGTCAGAAGATGAAGAG  480
HisIleSerGlyGlnHisLeuValAlaValGluGluAspAlaGluSerGluAspGluGlu  129
 * * * * * *----------------P-peptide-------------Asp

GAGGAGGATGTGAAACTCTTAAGTATATCTGGAAAGCGGTCTGCCCCTGGAGGTGGTAGC  540
GluGluAspValLysLeuLeuSerIleSerGlyLysArgSerAlaProGlyGlySer     149
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AAGGTTCCACAGAAAAAAGTAAAACTTGCTGCTGATGAAGATGATGACGATGATGATGAA  600
LysValProGlnLysLysValLysLeuAlaAlaAspGluAspAspAspAspAspAspGlu  169
       * * * * * *

GAGGATGATGATGAAGATGATGATGATGATGATTTTGATGATGAGGAAGCTGAAGAAAAA  660
GluAspAspAspGluAspAspAspAspAspAspPheAspAspGluGluAlaGluGluLys  189

GCGCCAGTGAAGAAATCTATACGAGATACTCCAGCCAAAAATGCACAAAAGTCAAATCAG  720
AlaProValLysLysSerIleArgAspThrProAlaLysAsnAlaGlnLysSerAsnGln  209
    * * * * * * *

AATGGAAAAGACTCAAAACCATCATCAACACCAAGATCAAAAGGACAAGAATCCTTCAAG  780
AsnGlyLysAspSerLysProSerSerThrProArgSerLysGlyGlnGluSerPheLys  229
                                    ---------

AAACAGGAAAAAACTCCTAAAACACCAAAAGGACCTAGTTCTGTAGAAGACATTAAAGCA  840
LysGlnGluLysThrProLysThrProLysGlyProSerSerValGluAspIleLysAla  249
-------------------------------------------------------------

AAAATGCAAGCAAGTATAGAAAAAGGTGGTTCTCTTCCCAAAGTGGAAGCCAAATTCATC  900
LysMetGlnAlaSerIleGluLysGlySerLeuProLysValGluAlaLysPheIle     269
-------------------------Antigenic peptide P42---------------

AATTATGTGAAGAATTGCTTCCGGATGACTGACCAAGAGGCTATTCAAGATCTCTGGCAG  960
AsnTyrValLysAsnCysPheArgMetThrAspGlnGluAlaIleGlnAspLeuTrpGln  289
-------------------------------------------------------------

TGGAGGAAGTCTCTTTAAGAAAATAGTTTAAACAATTTGTTAAAAAATTTTCCGTCTTAT 1020
TrpArgLysSerLeu-COOH
---------------

TTCATTTCTGTAACAGTTGATATCTGGCTGTCCTTTTTATAATGCAGAGTGAGAACTTTC 1080
CCTACCGTGTTTGATAAATGTTGTCCAGGTTCTATTGCCAAGAATGTGTTGTCCAAAATG 1140
CCTGTTTAGTTTTTAAAGATGGAACTCCACCCTTTGCTTGGTTTTAAGTATGTATGGAAT 1200
GTTATGATAGGACATAGTAGTAGCGGTGGTCAGACATGGAAATGGTGGGGAGACAAAAAT 1260
ATACATGTGAAATAAAACTCAGTATTTTAATAAAGTAAAAAAAAAAAAAAA           1311
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FIGURE 2: cDNA and amino acid sequences of nucleophosmin. The peptide sequence of rat nucleophosmin is indicated by dashed lines. The consensus nucleotide sequence for translation initiation (GCCGCCACC), the initiation codon (ATG), the stop codon (TAA), and the polyadenylation signal (AATAAA) are in boldface letters. The acidic amino acid clusters are also in boldface letters and underlined. The possible metal binding site and two karyophilic sequences are marked with an asterisk.

the phosphopeptide, and T1B were previously reported (Jones, 1979; Chan et al., 1986a,b). All five peptide sequences of nucleophosmin (1–5 of Table I) matched the cDNA-encoded amino acid sequence (Figure 2, dashed line). One exception is the 15th amino acid of the phosphopeptide in which Glu (instead of Asp) is in human nucleophosmin.

The cDNA (hPB6) encodes a total of 294 amino acids (including the first Met) with a calculated molecular mass of 32 578 daltons. The predicted amino acid composition of the cDNA-encoded nucleophosmin (Table II) correlates very well with that of the experimental results (Jones, 1979). Since N-terminal analysis and sequencing from the N-terminal of nucleophosmin did not yield any amino acid, the N-terminal may be blocked.

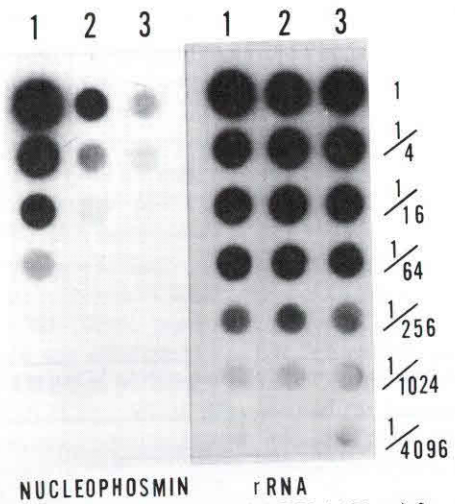

FIGURE 3: Dot blot analysis. Cellular RNA (30 μg) from Novikoff hepatoma cells (1), thioacetamide-treated rat liver cells (2), and normal rat liver cells (3) was serially diluted 4-fold and blotted on nitrocellulose membranes, which were then hybridized with ^{32}P-labeled nucleophosmin cDNA and genomic rDNA (18S + 28S) probes.

Table I also presents compositions of other specific domains within the amino acid sequence of nucleophosmin. The most hydrophilic region of the nucleophosmin molecule consists of two acidic amino acid clusters in the middle of the molecule. The first cluster, located at amino acids 120–132, contains 11 Asp/Glu and 1 Ser and Ala. The second cluster, located at 161–188, contains 26 Asp/Glu and 1 Phe and Ala. The phosphorylation site sequence, S(p)EDEEEED, is in the first acidic cluster. The sequence from amino acids 104 to 115 with one Cys (in position 104) and two His (in positions 110 and 115) has the structure of a potential metal binding site according to Lee et al. (1987). There are also two potential karyophilic sequences (Kalderon et al., 1984) located at amino acids 152–157 and 191–197, flanking the second acidic cluster.

Nucleophosmin mRNA Level in Normal versus Abnormal Liver. Equal amounts of total cellular RNA from Novikoff hepatoma, thioacetamide-treated hypertrophic liver, and normal liver cells were dotted onto nitrocellulose membranes and hybridized with different DNA probes (nucleophosmin and rRNA). Figure 3 shows the autoradiogram of the dot blot after hybridization. Since the quantity of rRNA applied in all three times was comparable, approximately equal amounts of cellular RNA were applied. From the relative intensity of the hybridization signals, there is more nucleophosmin mRNA in tumor cells than in normal liver. Quantitation of the relative levels of radioactivity of the dots showed that the level of nucleophosmin mRNA in normal rat liver is equal to the 1:4 and 1:64 dilutions of that in hypertrophic liver and Novikoff hepatoma cells, respectively.

Northern Blot Analysis. A single mRNA band hybridizing with the nucleophosmin cDNA probe was observed in the Northern blot of Novikoff hepatoma, hypertrophic rat liver, and normal rat liver (Figure 4). The size of nucleophosmin mRNA was estimated to be about 1600 bp. The ratio of nucleophosmin mRNA levels was 50:5:1 in Novikoff hepatoma, hypertrophic liver, and normal liver, as determined by the levels of radioactivity of the hybridizing bands.

Nucleophosmin Protein Level in Normal versus Abnormal Liver. Nuclear protein samples (containing equal amounts of DNA) from Novikoff hepatoma, hypertrophic rat liver, and normal rat liver were separated by 10% SDS–PAGE and subsequently analyzed by Western blot immunoassay. Figure 5 shows the autoradiogram of Western blot analysis. The immunoband of nucleophosmin was present in all three tissues.

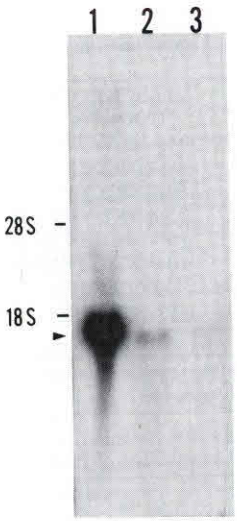

FIGURE 4: Northern blot analysis. Equal amounts of cellular RNA (10 μg) from Novikoff hepatoma cells (1), thioacetamide-treated rat liver cells (2), and normal rat liver cells (3) were separated by formaldehyde/1% agarose gel electrophoresis. The aliquots of RNA were then blotted onto a nylon membrane and hybridized with ^{32}P-labeled nucleophosmin cDNA probe.

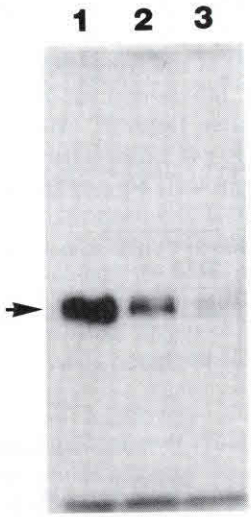

FIGURE 5: Western blot analysis. Aliquots (containing 70 μg of DNA) of nuclear protein from Novikoff hepatoma cells (1), thioacetamide-treated rat liver cells (2), and normal rat liver cells (3) were dissolved in 1% SDS/5 M urea/Laemmli sample buffer (pH 6.8). The nuclear proteins were separated with SDS–PAGE and immunostained with monoclonal anti-nucleophosmin antibody.

The ratio of nucleophosmin in Novikoff hepatoma, hypertrophic rat liver, and normal rat liver was 20:5:1, as determined by the levels of ^{125}I radioactivity in the immunobands.

Discussion

Characterization of the cDNA and Amino Acid Sequence of Nucleophosmin. The most striking feature of the amino acid sequence of nucleophosmin is the stretch of 26 acidic amino acids (Asp/Glu) in the middle portion of the sequence. Acidic amino acid clusters have been observed in a number of proteins especially nuclear proteins (Burton et al., 1981 Walker, 1982; Van Loon et al., 1984; Erickson et al., 1985; Pentecost et al., 1985; Reynolds et al., 1985; Stanton et al., 1986; Dingwall et al., 1987; Earnshaw, 1987; Kang et al., 1987; Lapeyre et al., 1987). Several proteins demonstrated a high degree of homology within the acidic amino acid cluster with nucleophosmin. Table III shows the comparison of the acidic acid amino cluster of proteins having more than 10 identical residues. The function of the acidic cluster has not been

Table III: Comparison of Acidic Amino Acid Clusters[g]

Proteins	Amino Acid #	Sequence
Nucleophosmin	161–188	D E D D D D D D E E D D D E D D D D D D F D D E E
HMG-T (Trout)	149–172	- D - - - - - - D D - - E D - - - - - - E - - -[a]
Ubiquinol-Cytochrome c reductase 17kd protein (S. cerevisiae)	46–73	- N G - E - E D - - E - - - - - - - - D E - - -[b]
RAD 6 protein (S. cerevisiae)	150–170	E - - M - D M - - - D - - - - - - D - - -[c]
RNA Polymerase (E. coli) sigma subunit	188–209	E - L - - - - D E - E - - G - - - S A - - D[d]
RNA Polymerase (S. typhimurium) sigma subunit	190–211	- - L - - - E D - - E E D G - - - A A D - -[e]
Amyloid A4 protein precursor (Human)	238–257	E E E - A - - - - - - E - G - E V E - -[f]

[a] Pentecost et al. (1985). [b] Van Loon et al. (1984). [c] Reynolds et al. (1985). [d] Burton et al. (1981). [e] Erickson et al. (1985). [f] Kang et al. (1987). [g] Dashes indicate that the amino acid is identical with that of nucleophosmin.

defined. It has been suggested that the negatively charged cluster might serve to confine the proteins inside the nucleus (Earnshaw, 1987). Another possible function is for binding with basic proteins, such as ribosomal proteins. It is noted that the phosphorylation site is in one of these acidic clusters of nucleophosmin. Phosphorylation of this site would enhance the local negative charge and thus the binding of basic proteins or other cations. It might also affect the conformation of the protein. Nucleophosmin is associated with RNPs in the nucleolus (Yung & Chan, 1987; Prestayko et al., 1974). During the assembly process of ribosomes, nucleophosmin is released from RNPs before ribosomes mature. Whether phosphorylation at this acidic cluster plays a role in the binding of nucleophosmin to RNP is not known.

The amino acid sequences of five peptides (comprising 138 amino acid residues) from rat nucleophosmin have been determined (Table I). They match the human cDNA-derived amino acid sequence. The assignment of the ATG at nucleotides 94–96 as the translation initiation codons is supported by the following observations: (1) The consensus sequence, GCCGCCACC, for the initiation of translation in vertebrate mRNAs (Kozak, 1987) is located at nucleotides 83–91, and is 2 bp upstream from this codon. (2) The amino acid composition derived from the cDNA sequence is in agreement with experimental results reported (Table II). (3) The predicted N-terminal sequence of human nucleophosmin (MEDSMDMD) is identical with that of *Xenopus* nucleophosmin (NO38) (Schmidt-Zachmann et al., 1987). It has been shown that the in vitro translation product of the cloned cDNA comigrated with native NO38 in 2D gel analysis. (4) The majority of blocked N-termini in proteins is due to acetylations of Ser and Ala residues (Persson et al., 1985). Since the N-terminus of nucleophosmin is blocked, there is a possibility that Met at position 9 might be a potential initiator codon and the N-terminal residue is Ser at position 10. This possibility, however, is unlikely due to the fact that the percentage composition of Met and Asp in the predicted protein will then deviate significantly from that obtained by amino acid composition analysis of the pure protein (Jones, 1979) (Table II). Besides, Met has also been reported to be the third most common N-terminal residue in N-acetylated proteins, especially those of the eukaryotic system (Persson et al., 1985).

The human nucleophosmin cDNA analyzed (hPBb) has 1311 bp, while Northern blot analysis of rat liver nucleophosmin showed a mature mRNA of about 1600 bp. This discrepancy in size suggests either that the cDNA is not full-length and there are about 200 bp missing in the 5′-nontranslated sequence or that human and rat nucleophosmin mRNAs albeit highly similar with respect to their coding sequence, have different sizes. The difference in size could be due to a difference in the length of either the 3′- or the 5′-nontranslated sequence or both.

There are more than one species of nucleophosmin present in human tissues. The α, β, and γ monomers of nucleophosmin have been identified by 2D gel analysis (Chan et al., 1985). Recent studies indicated that nucleophosmin is a hexamer consisting of four α and two β monomers (Yung & Chan, 1987). Two highly homologous but not identical partial cDNAs of nucleophosmin in human placenta have been reported (Chan et al., 1986b). Both cDNAs (hPB1 and hPB2) encode the C-terminal 82 amino acids of nucleophosmin and the 3′-nontranslating sequence (Chan et al., 1986b). hPB1 and hPB2 are almost identical except for six nucleotide changes just upstream of the immunoreactive region. Alternation of these six nucleotides causes change in four amino acids. The cDNA reported in the present study corresponds to hPB2. Several other nucleophosmin cDNA clones were identified from human testis and HeLa cell libraries. Nucleotide sequences of these partial cDNAs showed that they are identical with that of hPB2 (results not shown). The calculated molecular mass of the cDNA-derived nucleophosmin is 32 578 daltons, which is smaller than the 37 000 daltons estimated by SDS gel electrophoresis. Unusual localization of the charged amino acids and the tertiary structure might retard the mobility of nucleophosmin in SDS gel electrophoresis (Schmidt-Zachmann et al., 1987; Lapeyre et al., 1987; Weber & Osborn, 1975), resulting in an apparent larger molecular mass.

Association of Nucleophosmin with Tissue Growth. Using the human nucleophosmin cDNA as probe, it is found that the relative amount of nucleophosmin mRNA is 50-fold more in tumor than in normal rat liver; the level in hypertrophic rat liver is also higher. Higher levels of mRNA of some oncogenes are often observed in hypertrophic or tumor tissues. For instance, transcription of ras^H and c-myc cellular oncogenes increases 5-fold during liver regeneration (Fausto & Shank, 1983). The transcription of c-myc increases 5–10-fold in sarcomas and carcinomas (Eva et al., 1982), and the tran-

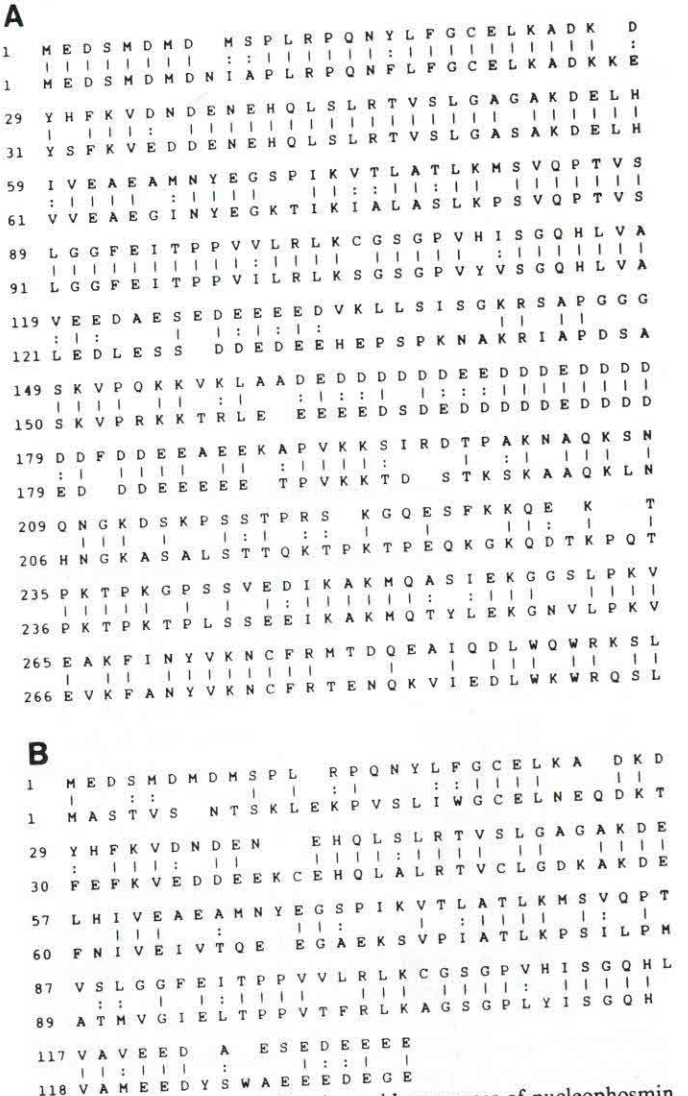

FIGURE 6: Comparison of amino acid sequences of nucleophosmin with NO38 and nucleoplasmin. (A) Comparison with NO38. (Vertical lines) Identical match; (colons) conservative exchange. Matches = 193, mismatches = 97, unmatched = 14, length = 299, matches/length = 64.5%. (B) Comparison with nucleoplasmin. (Vertical lines) Identical match; (colons) conservative exchange. Matches = 67, mismatches = 61, unmatched = 7, length = 135, matches/length = 49.6%.

scription of c-ras^H increases 2.5–15-fold in malignant breast carcinomas (Spandidos & Agnantis, 1984). The present study shows that the relative amounts of nucleophosmin protein and mRNA in tumor cells are about 20 times and 50 times higher, respectively, than in normal liver cells. Even though neither the stability of nucleophosmin mRNA nor the transcriptional efficiency of the nucleophosmin gene in the tumor and normal systems has been examined in the present study, it is apparent that change in the overall synthesis of nucleophosmin during neoplastic growth is more significant than that of c-myc and c-ras^H oncogenes. The presence of a translational control mechanism for nucleophosmin is also apparent as revealed by the difference seen with the protein and mRNA levels in Novikoff hepatoma cells. This difference is unlikely to be caused by experimental variation since multiple experiments consistently showed a 50-60-fold increase in mRNA level and only an 18-20-fold increase in protein level in Novikoff hepatoma cells.

Studies of Sequence Homology and Function of Nucleophosmin. The results presented suggest a high degree of homology existing between human and rat nucleophosmin. Comparison of human nucleophosmin and *Xenopus* nucleolar protein NO38 (Schmidt-Zachmann et al., 1987) shows 64.3% sequence identity between the two proteins (Figure 6A). These results indicate that nucleophosmin has been well conserved during evolution and might play an important role in some basic cellular activity such as ribosome synthesis. The N-terminal 130 amino acids of human nucleophosmin (Figure 6B) have 50% similarity with the N-terminal 130 amino acids of *Xenopus* nucleoplasmin (Dingwall et al., 1987). The hydrophobicity and the secondary structure of the N-terminal of the two proteins are very similar (data not shown). Since the function of nucleoplasmin appears to be assistance of histone assembly into nucleosomes (Earnshaw et al., 1980; Dingwall et al., 1987; Laskey et al., 1978), it is reasonable to assume that nucleophosmin is responsible for assisting ribosomal protein assembly in preribosomal particles in an analogous manner.

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Registry No. DNA (human nucleophosmin mRNA complementary), 117896-07-8; nucleophosmin (human protein moiety reduced), 117896-08-9.

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