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A 10S Particle Released from Deoxyribonuclease-Sensitive Regions of HeLa Cell Nuclei Contains the 86-Kilodalton–70-Kilodalton Protein Complex†

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ABSTRACT: Digestion of HeLa cell nuclei with micrococcal nuclease or deoxyribonuclease I (DNase I) released the 86-kilodalton–70-kilodalton (kDa) protein complex in particles sedimenting at approximately 10 S in sucrose density gradients. Immunoaffinity-purified ^{32}P-labeled complexes contained 86- and 70-kDa polypeptides with phosphorylated serine residues and DNA fragments, of which the largest was 110 base pairs long. Digestion of nick-translated nuclei with micrococcal nuclease released ^{32}P-labeled 10S particles that were immunoaffinity-purified; they contained labeled 110-base-pair DNA fragments. The micrococcal nuclease digests were analyzed by two-dimensional electrophoresis, which separated nucleosomes in the first dimension and the associated proteins in the second. Western blots of the separated proteins showed that the 86-kDa–70-kDa complex was associated with the mono-, di-, and trinucleosomes. A more extensive electrophoretic separation revealed that the 10S particle from nick-translated nuclei migrated with a subfraction of the mononucleosomes that lacked H1 histones. These results suggest that the 10S particle which contains the 86-kDa–70-kDa complex is associated with an unfolded nucleosome that is present in DNase I sensitive regions.

Recently, monoclonal antibodies were developed in our laboratory that recognized a polypeptide of M_r 86 000 and a p*I* 6.0 (Yaneva et al., 1985a). With these antibodies, the antigen was immunoaffinity-purified and shown to be in a complex of two polypeptides of M_r 86 000 and M_r 70 000. The release of this complex by treatment of nuclei with DNase I and the reduction of nucleolar immunofluorescence by treatment with the DNase I suggested that this antigen was a DNA-binding nonhistone protein (Yaneva et al., 1985a). Preliminary immunofluorescence studies with synchronized HeLa cells showed cell cycle variations in the pattern of distribution of the antigen within the nucleus and nucleolus (Zweig et al., 1984). Reeves (1985) has recently identified a similar antigen localized to the nucleus with monoclonal antibodies to HeLa nuclei. This antigen is a DNA-binding, nonhistone protein that is recognized by a number of sera from patients with autoimmune diseases (systemic lupus erythematosus, mixed connective tissue diseases, and scleroderma). An antigen that binds selectively to DNA and consists of 80- and 70-kDa[1] polypeptides was also found by Mimori et al. (1985), who reported that it was recognized by autoimmune sera from patients with rheumatic disorders. It is possible these studies all deal with similar antigens.

This study was done to learn whether the 86-kDa–70-kDa complex was in a particle and if it relates to nucleosomal structures. Although DNA and histones form the basic nucleosome structure (McGhee & Felsenfeld, 1980; Kornberg & Klug, 1981), not much is yet clear about the presence of nonhistone proteins. It has been suggested that nonhistone proteins maintain a special unfolded conformation of the nucleosome to permit transcription and replication (Weisbrod, 1982; Levinger & Varshavsky, 1982); a consequence of this altered conformation is increased DNase I sensitivity of these regions of the chromatin (Weisbrod & Groudine, 1980; Weintraub, 1985). It has been shown (Gazit et al., 1980) that proteins extracted from the cell nucleus with 0.35 M NaCl, particularly HMG 14 and 17, confer DNase I sensitivity (Weisbrod & Groudine, 1980; Weisbrod, 1982a,b). A role

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[1] Abbreviations: kDa, kilodalton(s); Tris, tris(hydroxymethyl)-aminomethane; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; PMSF, phenylmethanesulfonyl fluoride; ELISA, enzyme-linked immunoabsorbent assay; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

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in loosening the chromatin structure was also proposed for ubiquitinated histone H2A (protein A_{24}) (Goldknopf & Busch, 1978; Levinger & Varshavsky, 1982; Busch, 1984).

This study shows that the 86-kDa–70-kDa complex, which can be dissociated from DNA with 0.35 M NaCl, is bound to a particle that migrates as a subfraction of the mononucleosomes and originates from the DNase I sensitive regions of the cell nucleus.

Materials and Methods

HeLa S-3 cells (1 g) were labeled in vivo by incubation for 16 h with 50 mCi of [^{32}P]orthophosphate in phosphate-free tissue culture medium formula no. 82-5104 (Gibco). Nuclei were isolated as described previously (Yaneva et al., 1985a).

Micrococcal Nuclease Digestion. The isolated nuclei were suspended in 10 mM Tris-HCl (pH 7.8)/1 mM $CaCl_2$/1 mM PMSF/5 μg/mL leupeptin/5 μg/mL aprotinin at a concentration of 2 mg/mL DNA (determined in 1% SDS). Micrococcal nuclease (P-L Biochemicals) digestion was performed at room temperature for 15 min with 40–300 units/mL enzyme. The reaction was stopped by adding EDTA and EGTA to a final concentration of 5 mM each. The nuclei were lysed for about 10 min on ice and centrifuged for 10 min at 12000*g*. The supernatant was analyzed on sucrose density gradients or by polyacrylamide gel electrophoresis. The degree of the digestion was determined by measuring absorbance at 260 nm of the nucleic acids soluble in 0.65 N $HClO_4$.

Nick Translation of HeLa Nuclei. The procedure used was similar to that described by Javaherian and Fasman (1984), originally proposed by Levitt et al. (1979). Nuclei were suspended in 50 mM Tris-HCl (pH 7.6)/5 mM $MgCl_2$/5 mM 2-mercaptoethanol/1 mM PMSF/50 μg/mL bovine serum albumin at a concentration of 2 mg/mL DNA (determined in 1% SDS). Nicking of the DNA was carried out by DNase I (Sigma) at a concentration of 0.5 μg/mL for 20 min at 37 °C; then the sample was placed on ice. The deoxynucleotides dATP, dGTP, dTTP, and dCTP (Sigma) containing 10 mCi of either [^{3}H]dTTP (New England Nuclear; 80 Ci/mmol) or [^{32}P]dCTP (Amersham, 400 Ci/mmol) were added to final concentration 8 μM of each. The reaction mixture was incubated with 20 units/mL *Escherichia coli* DNA polymerase I (New England Biolabs; 10 000 units/mL) for 10 min at 15 °C. After the reaction the nuclei were centrifuged and washed 3 times with the suspension buffer.

Sucrose Density Gradients. Linear sucrose density gradients 5–20% in 10 mM Tris-HCl (pH 8.0)/1 mM EDTA/0.1 mM PMSF were used for analysis of the soluble oligonucleosomes. The centrifugation was performed in 13-mL tubes in a Beckman SW-41 rotor at 4 °C for 20 h at 32 000 rpm.

Gel Electrophoresis. Low ionic strength electrophoresis in 5% polyacrylamide gels (3 mm thick and 30 cm long) for separation of the nucleosomes was performed according to Levinger and Varshavsky (1980). The buffer in the gel and electrode chambers was 6.4 mM Tris–base/3.2 mM sodium acetate/4 mM EGTA/0.5 mM EDTA (pH 8.0) as described (Javaherian & Fasman, 1984). Preelectrophoresis was carried out at 150 V (~25 mA) for 1.5 h. The electrophoresis was run at 4 °C for 24 h at 150 V. The electrode buffer was recirculating between the two reservoirs. For protein analysis, 2-cm strips of polyacrylamide gel from the first dimension were incubated for 2 h with shaking in Laemmli sample buffer containing 2.3% SDS/0.0625 Tris-HCl (pH 6.8)/5% 2-mercaptoethanol.

The second-dimension protein analysis was performed on either 8% (for Western blot analysis) or 15% (for silver staining of the proteins) SDS–polyacrylamide gels 3 mm thick (Laemmli, 1970). The electrophoresis was run at 150 V. Proteins were stained with silver (Oakley et al., 1980).

Nucleic acids were extracted with phenol after extensive digestion with proteinase K (200 μg/mL, at 37 °C overnight) and after ethanol precipitation were analyzed on a 10% polyacrylamide gel with 0.09 M TBE buffer (Tris/boric acid/EDTA) according to Maniatis et al. (1975).

Immunological Assays. The ELISA and immunoblot assays were performed with anti-86- or anti-70-kDa monoclonal antibodies from hybridoma cell culture supernatants as described previously (Yaneva et al., 1985a). The monoclonal antibodies were produced as described earlier (Freeman et al., 1985). Rabbit anti-topoisomerase I antibodies were a gift from Dr. E. Durban.

Salt Extraction of 86-kDa–70-kDa Complex. Isolated HeLa cell nuclei at a concentration of 4×10^8/mL (2 mg of DNA/mL) were homogenized in 10 mM Tris-HCl (pH 8.0)/1 mM PMSF/5 μg/mL leupeptin/5 μg/mL aprotinin and extracted at 4 °C for 20 min. The suspension was centrifuged for 10 min at 10000*g* and the pellet was reextracted with the same buffer. The combined supernatants were analyzed for the 86-kDa–70-kDa complex by immunoblots. The pellet was reextracted sequentially with the same buffer containing increasing concentrations of NaCl–0.35, 0.6, and 1 M. All supernatants were analyzed for 86-kDa–70-kDa protein by immunoblots with the anti-86-kDa monoclonal antibody.

Phosphoamino Acid Analysis. After immunoaffinity purification of 86-kDa–70-kDa from HeLa cell nuclei labeled in vivo with [^{32}p]orthophosphate, the proteins were separated on 8% SDS gels. The gels were sliced to cut out the 86- and 70-kDa proteins that were digested with trypsin (500 μg/mL, overnight at 37 °C); the peptides were hydrolyzed with 6 N HCl for 2 h at 100 °C. The amino acids were separated by high-voltage paper electrophoresis at pH 3.5 (Ahn et al., 1985).

Results

Digestion of HeLa Nuclei with Micrococcal Nuclease. Treatment of HeLa nuclei with micrococcal nuclease progressively released the 86-kDa–70-kDa antigen from the nucleus (Figure 1). The main peak of the antigen migrated in the 10S region in a 5–20% sucrose density gradient. The antigen was also detected at the 13S region between the mono- and dinucleosomal peaks. The extent of release of the antigen depended on extent of nuclease digestion (Figure 1B–D); the 10S peak increased with increasing digestion. For the complete extraction of the antigen from the cell nucleus, DNase I digestion was employed (Figure 2 and Yaneva et al., 1985a). This result was reproducibly obtained with several different monoclonal antibodies to the 86- or 70-kDa polypeptides.

After HeLa cells were labeled in vivo with [^{32}P]orthophosphate, the isolated nuclei were digested with micrococcal nuclease; the labeled ELISA peak at 10S in the sucrose density gradient was collected and immunoaffinity-purified as described earlier (Yaneva et al., 1985a). The complex contained ^{32}P-labeled 86- and 70-kDa polypeptides, which were both labeled at serine residues (Figure 3), and DNA and RNA fragments. Figure 4A shows the electrophoretic profile of the nucleic acid components derived after the protein was digested with proteinase K (210 μg/mL enzyme, at 37 °C overnight); the sample was extracted with phenol and precipitated with ethanol. The three DNA fragments of 110, 90, and 72 base pairs were similar in size to the DNA fragments obtained from the immunoaffinity-purified antigen from Namalwa cell nuclei (Yaneva et al., 1985b). These DNA fragments are apparently protected by the 86-kDa–70-kDa protein against micrococcal nuclease attack and may represent the binding sites of the

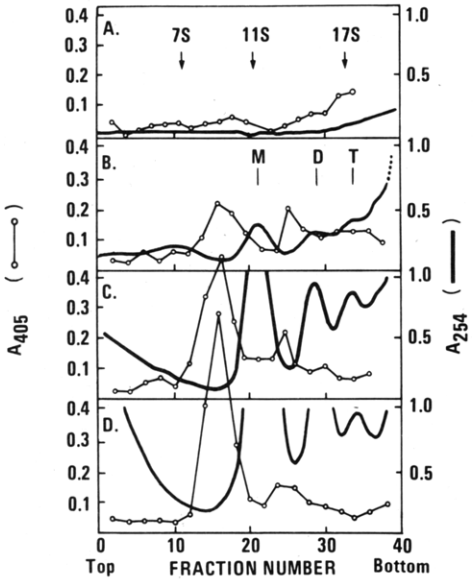

FIGURE 1: Sucrose density gradient profiles of nucleosomal preparations. Supernatants with soluble oligonucleosomes after micrococcal nuclease digestion of nuclei were loaded on 5–20% sucrose gradients. After centrifugation, 0.3-mL fractions were collected and assayed by the ELISA assay for the presence of 86-kDa–70-kDa antigen (O). M, D, and T mark the position of mono-, di-, and trinucleosomes, respectively. The gradients were calibrated with catalase (7 S), lactate dehydrogenase (11 S), and apoferritin (17 S). (A) No enzyme, 1.3% acid-soluble material; (B) 40 units/mL enzyme, 4.2% acid-soluble material; (C) 100 units/mL enzyme, 7.0% acid-soluble material; (D) 300 units/mL, 9.1% acid-soluble material.

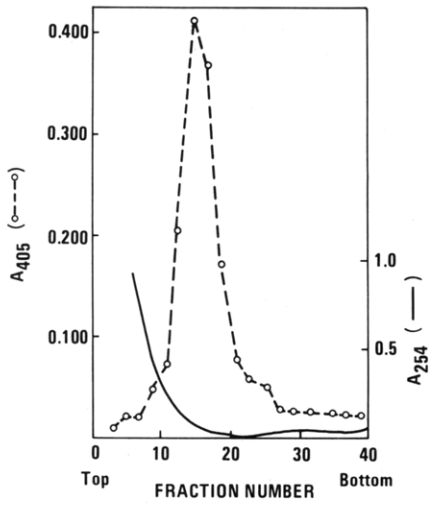

FIGURE 2: Sucrose density gradient of the soluble material released from HeLa nuclei after digestion with DNase I. HeLa nuclei at a concentration of about 4×10^8/mL were digested with 50 μg/mL DNase I for 30 min on ice in the presence of 1 mM $MgCl_2$ and protease inhibitors—1 mM PMSF and leupeptin and aprotinin at 5 μg/mL each—at pH 7.8. The reaction was stopped by adding EDTA to 2 mM, the mixture was centrifuged for 10 min at 10000g, and the clear supernatant was applied to a 5–20% sucrose density gradient. After centrifugation the fractions were analyzed for 86-kDa–70-kDa complex by ELISA (O) using anti-86-kDa monoclonal antibody.

complex to DNA. The associated RNA was heterogeneous and small, which reflects its degradation by micrococcal nuclease. By comparison, the 10S ELISA peak obtained after digestion with DNase I did not contain discrete DNA fragments after immunoaffinity purification.

To determine if the antigen was bound to the DNase I sensitive regions in the nucleus, the method of Levitt et al. (1979) was used to label DNase I sensitive regions. Micrococcal nuclease digestion of nick-translated nuclei also released

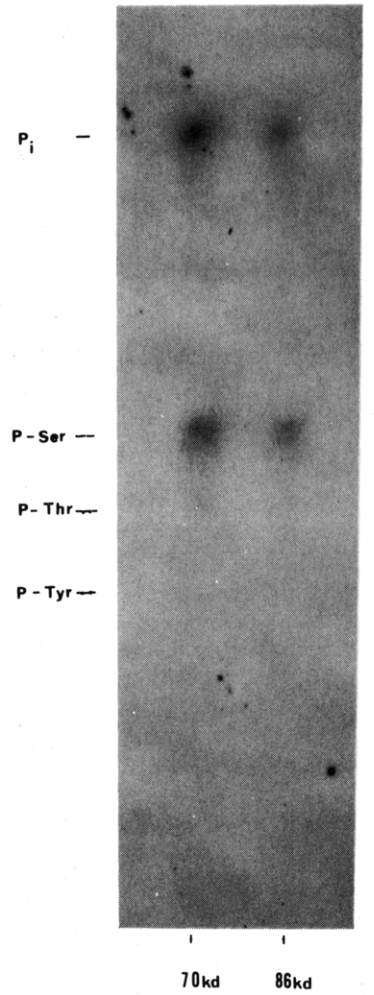

FIGURE 3: Phosphoamino acid analysis of 86- and 70-kDa polypeptides. In vivo ^{32}P-labeled proteins were immunoaffinity-purified, separated, extracted, hydrolyzed, and subjected to electrophoresis at pH 3.5 as described under Materials and Methods. Positions of markers phosphoserine, phosphothreonine, phosphotyrosine, and inorganic phosphate are indicated on the autoradiogram.

the antigen at 10S in the sucrose density gradient comigrating with a small peak of ^{32}P-labeled nick-translated DNA [Figure 5; see Javaherian & Fasman (1984)]. The low molecular weight material on top of the gradient contained 47% of the ^{32}P label, 25% was associated with the ELISA peak at 10S, and 18% and 10% comigrated with the mono- and dinucleosomal peaks, respectively. The 10S peak was immunoaffinity-purified and the protein was digested; the nick-translated DNA was extracted with phenol, precipitated with ethanol, and electrophoresed on a 10% gel (Figure 4B). A DNA fragment approximately 110 base pairs long was found along with some low molecular weight degraded fragments. The 110-base-pair DNA fragment associated with the immunoaffinity-purified 86-kDa–70-kDa complex probably originates from the DNase I sensitive regions in the chromatin.

Localization of Topoisomerase I in the Sucrose Density Gradient. Since it has been shown (Weisbrod, 1982a,b; Weintraub, 1985; Bonven et al., 1985) that topoisomerase I is associated with DNase I hypersensitive sites of the actively transcribed genes, anti-topoisomerase I antibody was used to localize this enzyme in the sucrose density gradients. The ELISA activity for topoisomerase I showed peaks in the 11S and 17S regions that correlated with mono- and dinucleosomal peaks, and some ELISA activity was associated with the degraded material on top of the gradient (Figure 5). The 10S ELISA peak for 86-kDa–70-kDa complex was between the

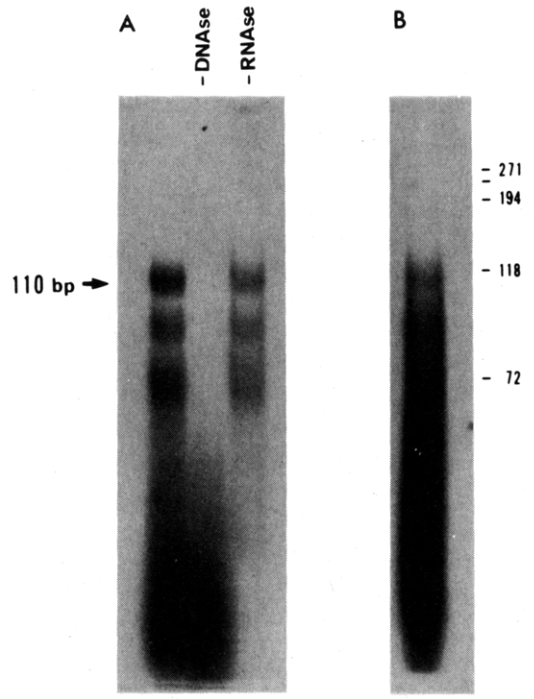

FIGURE 4: Electrophoresis of nucleic acids isolated from the immunoaffinity-purifed 86-kDa–70-kDa complex. (A) Nucleic acids isolated from the in vivo ^{32}P-labeled complex: lane 1, untreated nucleic acids; lane 2, those treated with 5 μg/mL DNase I for 30 min at 37 °C in the presence of 1 mM $MgCl_2$ at pH 7.8; lane 3, those treated with RNases T1 and A, 5 μg/mL of each, for 30 min at 37 °C. (B) Nucleic acids isolated from the immunoaffinity-purified complex derived from nick-translated nuclei in the presence of [^{32}P]dCTP HeLa nuclei. The numbers (in base pairs) represent the position of DNA fragments produced by digestion of ϕX174 DNA with *Hae*III restriction nuclease.

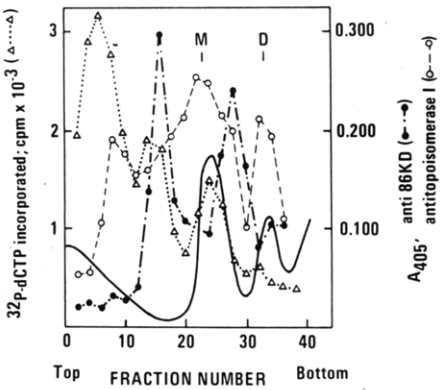

FIGURE 5: Sucrose density gradient of nucleosomes released by digestion of nick-translated HeLa nuclei with micrococcal nuclease. Nick translation and digestion of the nuclei were performed as described under Materials and Methods. The nuclei were digested to 7.5% acid-soluble material. The centrifugation was done as described under Materials and Methods and in the legend of Figure 1. The fractions across the gradient were counted after precipitation with trichloroacetic acid (Schleif et al., 1981) and assayed by ELISA with anti-86-kDa monoclonal antibody (●) and anti-topoisomerase I rabbit antibody (○). M and D denote the position of mono- and dinucleosomes.

peak of free topoisomerase (about 6S) and the mononucleosomes.

Two-Dimensional Analysis of the Nucleosomes. The first dimension of the two-dimensional analysis resolves the soluble oligonucleosomes (Levinger & Varsharsky, 1980). Figure 6A shows a portion of the ethidium bromide stained gel containing mono- and dinucleosomal fractions. Their positions were determined with mono- and dinucleosome material from the peaks in the sucrose density gradient. Mononucleosomes were distributed into three subfractions—a broad rapidly migrating

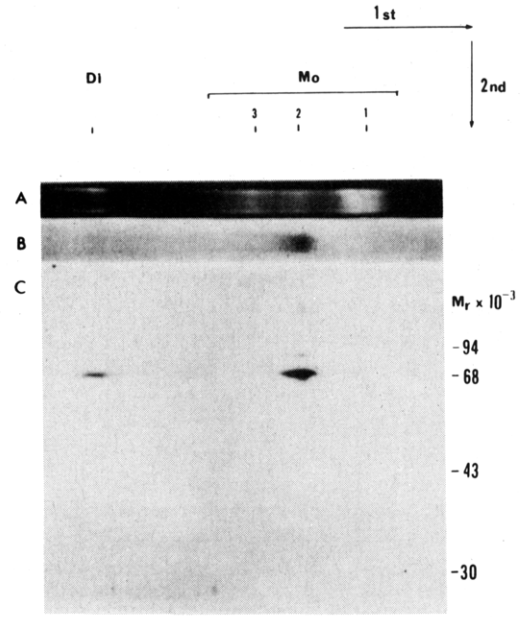

FIGURE 6: Strip A: Low ionic strength gel electrophoresis of the supernatant after digestion of HeLa nuclei with micrococcal nuclease. Nuclei were digested with 200 units/mL nuclease as described under Materials and Methods to about 10% acid-soluble material. The strip represents a portion of the ethidium bromide stained gel with mono- and dinucleosomal fractions designated Mo and Di. 1, 2, and 3 mark the positions of the subfractions of the mononucleosomes. Strip B: Autoradiography of an identical gel on which the 10S fraction from the sucrose density gradient after digestion of nick-translated nuclei in the presence of [^{32}P]dCTP nuclei was separated. Strip C: Immunoblot of the proteins migrating with the mono- and dinucleosomal fractions in the first dimension and separated by SDS electrophoresis in the second dimension. The proteins transferred onto nitrocellulose paper were sequentially stained with anti-86- and anti-70-kDa monoclonal antibodies.

fraction (fraction 1, Figure 6) and two slower migrating fractions (fractions 2 and 3, Figure 6)—as described in earlier reports (Javaherian & Fasman, 1984; Levinger & Varshavsky, 1980). Figure 6B shows the position of the ^{32}P-labeled fraction from the 10S peak from a sucrose density gradient of the micrococcal nuclease digestion of nick-translated nuclei. A labeled band migrated in the position of the slower migrating submononucleosomal fraction (fraction 2), which is in agreement with the results of Javaherian and Fasman (1984).

Mono- and dinucleosomal portions of the first-dimension gel were analyzed in the second dimension by immunoblots using both anti-86- and anti-70-kDa monoclonal antibodies. The antigen was mainly bound to the slower migrating subfraction of the mononucleosomes, labeled by nick translation (Figure 6C). In addition, the antigen was also found in the dinucleosomes. The difference in the intensity between 86- and 70-kDa spots (Figure 6C) is due to different titers of the two antibodies. The silver-stained SDS gels showed the two polypeptides were in a 1:1 weight ratio (Yaneva et al., 1985a; Figure 8A). When the oligonucleosomes were electrophoresed in the first dimension for shorter times so that mono-, di-, and trinucleosomes could be analyzed by Western blot in the second dimension, the 86-kDa–70-kDa complex was associated with all three fractions (Figure 7).

The proteins migrating with the mononucleosomal fractions were separated by SDS gel electrophoresis in the second dimension (Figure 8). The silver-stained 15% SDS gel shows two bands labeled with white arrows (Figure 8A), which are the immunoreactive 86- (Figure 8B) and 70-kDa polypeptides. Histone H1 was associated with the slowest migrating submononucleosomal fraction (fraction 3 in Figure 6A). The

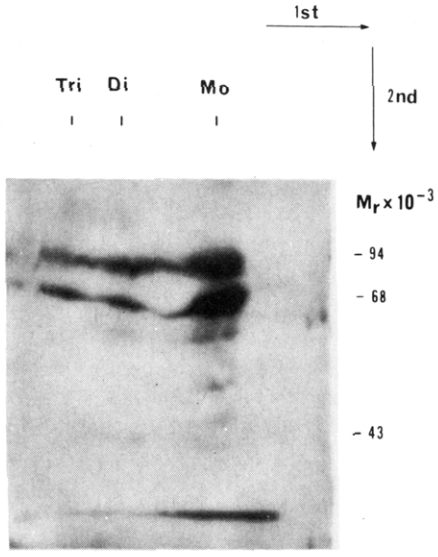

FIGURE 7: Immunoblot of proteins migrating with mono-, di-, and trinucleosomal fractions in the first dimension electrophoresis and separated on 8% SDS gel in the second dimension. The proteins were sequentially stained first with anti-86- and then with anti-70-kDa monoclonal antibodies. Mo, Di, and Tri designate the positions of mono-, di-, and trinucleosomal fractions in the first dimension electrophoresis.

black arrow in Figure 8A shows that a protein that comigrated with ubiquitinated histone H2A (protein A_{24}) was present in small amounts.

Extraction of 86-kDa–70-kDa Complex with 0.35 M NaCl. Gazit et al. (1980) have shown that DNase I sensitivity was reduced by washing the nuclei with 0.35 M NaCl and subsequently reconstituted by adding the extracted proteins back to the nuclei. To determine the optimal salt concentrations for extraction of the 86-kDa–70-kDa complex from the nucleus, HeLa nuclei were sequentially treated with solutions containing increasing concentrations of NaCl and the supernatants were analyzed for the presence of 86-kDa–70-kDa protein by immunoblots with the anti-86-kDa monoclonal antibody. Most of the 86-kDa–70-kDa complex, 55%, was extracted with 0.35 M NaCl (Figure 9) and 35% with 0.6 M NaCl. About 3% of the antigen was left in the residue after extraction with 1 M NaCl, which may represent very tightly bound DNA–protein complexes.

Discussion

The 86-kDa—70-kDa protein complex can be released from the cell nucleus by treatment with micrococcal nuclease, DNase I, or 0.35 M NaCl. Analysis of the released material after nuclear digestion demonstrates that the protein is associated with a 10S particle. A similar Svedberg value was reported by Reeves (1985) for the 80-kDa–70-kDa nuclear antigen released from HeLa nuclei by sonication. The discrete ELISA peaks in the sucrose density gradient as well as the association of the 86-kDa–70-kDa complex with di- and trinucleosomal fractions in the two-dimensional gel analysis suggest that the antigen is distributed on the nucleosomes along the chromatin fibers. The 10S particle contains DNA and the core histones but lacks histone H1 (Figure 8A). Thus, it is possible that this particle associates with unfolded nucleosomal structures; as a result, it migrates in a slightly different position than the bulk mononucleosomes in the sucrose density gradients. It may be similar to the particle Javaherian and Fasman (1984) observed in nick-translated HeLa nuclei.

The 10S particle has a complex protein composition since other nonhistone proteins cosediment with the 86-kDa–70-kDa

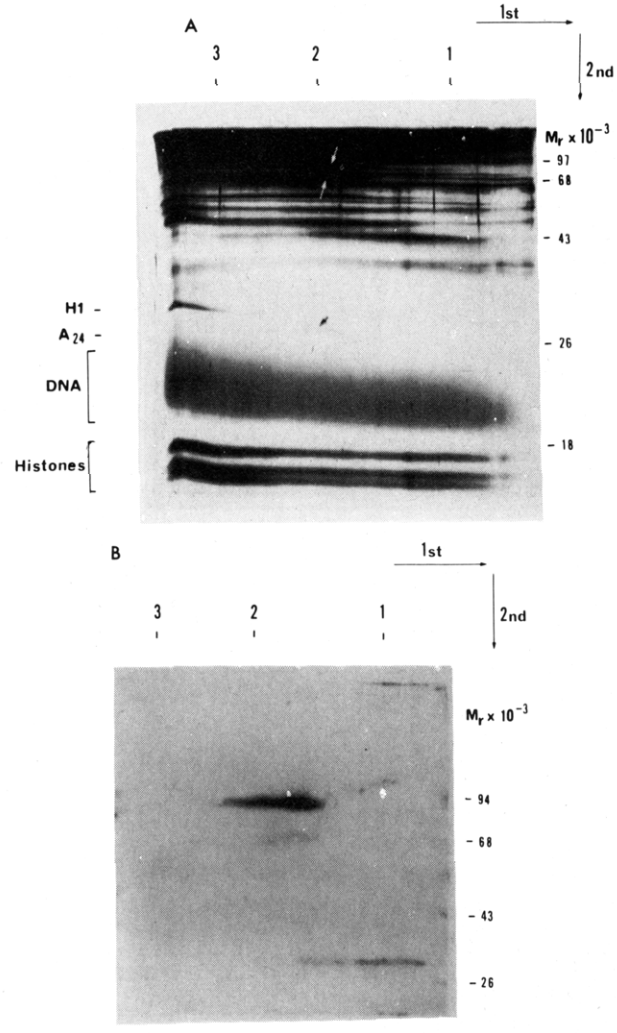

FIGURE 8: (A) Second dimension electrophoresis on 15% SDS gel of proteins migrating with the mononucleosomal fractions (1, 2, and 3 as in Figure 6A) in first dimension electrophoresis. The white arrows point to the position of 86- and 70-kDa polypeptides, respectively. The black arrow points to the protein that migrates with the mobility of protein A_{24}. The gel was stained with silver, which stains DNA as well. (B) Immunoblot of the same proteins after separation on 8% SDS gel stained with anti-86-kDa monoclonal antibody.

complex, including a protein with the mobility of protein A_{24} (Figure 8A). Other authors (Levinger & Varshavsky, 1980; Javaherian & Fasman, 1984) showed this mononucleosomal subfraction contains HMG proteins 14 and 17; protein A_{24} migrated slightly faster in the tail of the core-particle fraction. The silver-stained gel did not reveal HMG proteins that migrate at the position of silver-stained DNA. Future experiments with cross-linking of the proteins in the 10S particle should give information about the structural relation of the 86-kDa–70-kDa protein complex to the other proteins.

The 86-kDa–70-kDa complex is bound to DNA fragments originating from the DNase I sensitive regions in the nucleus with sizes 110, 90, and 72 base pairs. These DNA fragments are protected by the 86-kDa–70-kDa protein complex against nuclease digestion. This binding may be specific since Mimori et al. (1985) reported a selective binding to human DNA of a nuclear protein with characteristics similar to 86-kDa–70-kDa complex. Cloning and sequencing of these fragments are being done to determine the DNA sequence to which the protein binds.

The DNA fragments are not covalently bound to the protein. The complex is dissociated by SDS electrophoresis into the 86- and 70-kDa polypeptides and 110-, 90-, and 72-base-pair

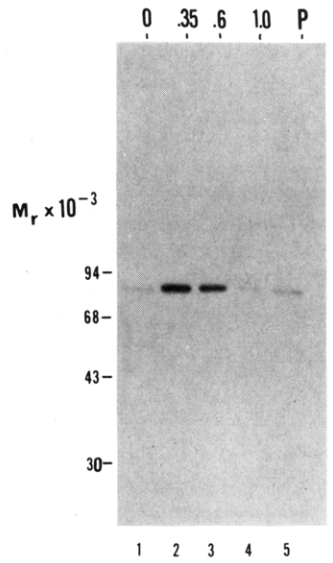

FIGURE 9: Immunoblot of proteins released from HeLa nuclei with different salt concentrations. The nuclei were suspended in 10 mM Tris-HCl (pH 8.0)/1 mM PMSF, incubated for 30 min on ice, and centrifuged. After the procedure was repeated the combined supernatants were applied to an 8% SDS gel. The pellet was extracted in the same manner with the same solution but containing increasing concentrations of NaCl. The electrophoretically separated proteins were blotted to nitrocellulose paper and stained with anti-86-kDa monoclonal antibody. Lane 1, no salt; lane 2, 0.35 M NaCl; lane 3, 0.6 M NaCl; lane 4, 1.0 M NaCl; lane 5, the pellet after 1 M NaCl solubilized with sample buffer.

DNA fragments (results not shown). Approximately 90% of the protein was dissociated from the DNA in the nucleus with 0.35 and 0.6 M NaCl (Figure 9). Since about 3% of the antigen remained in the pellet even after extraction of the nuclei with 1 M NaCl (Figure 9), a small population of the protein molecules may be very tightly, transiently bound to the DNA. Topoisomerase I is also extractable with 0.35 M NaCl, but some of it remains covalently bound to the DNA (Durban et al., 1981; Champoux, 1981).

The possibility that the DNA fragments found in the immunoaffinity-purified complex are nonspecifically bound as contaminations from degraded DNA seems unlikely because the DNA fragment pattern was reproducible. The 110-base-pair DNA fragment was consistently found in the purified complex, even after the immunoaffinity column was washed with 1 M NaCl (results not shown).

Digestion of nick-translated nuclei showed that the 10S particle originates from the DNase I sensitive regions in the chromatin. Actively transcribed DNA sequences in the nucleus are more sensitive to digestion with DNase I (Weintraub & Groudine, 1976; Weisbrod & Groudine, 1980; Weisbrod, 1982b; Weintraub, 1985) than the repressed sequences as a result of their more open conformation. These regions lack histone H1, which may be involved in gene repression (Weintraub, 1985). It has been suggested that portions of the active genes are organized in canonical nucleosomal structures (Lacy & Axel, 1975; Bellard et al., 1977; Weintraub, 1985); however, active ribosomal (McKnight et al., 1979; Labhart & Voller, 1982) and some nonribosomal (Anderson et al., 1980; Anderson et al., 1982) genes appear to be devoid of nucleosomes. During gene activation, nucleosomes may be converted to a more extended structure composed of an unfolded histone octamer and specific nonhistone proteins (Prior et al., 1983; Javaherian & Fasman, 1984). It is possible that the 86-kDa–70-kDa complex is one of the factors involved in this process.

The 10S particle, containing the 86-kDa–70-kDa complex, was released into the soluble fraction after digestion of nuclei with micrococcal nuclease. With DNA probes to specific genes, it has been shown (Cohen & Sheffery, 1985; Einck et al., 1985) that many actively transcribed sequences remain in the insoluble fraction after similar digestions; they are not associated with nucleosomes while their nontranscribed, flanking sequences are soluble and nucleosomes-associated (Cohen & Sheffrey, 1985). In our experiments, topoisomerase I was also released in the soluble fraction. Topoisomerase I is tightly associated with DNase I hypersensitive sites in the nontranscribed spacers flanking the rRNA genes in *Tetrahymena* (Bonven et al., 1985). Topoisomerase I may be involved in inducing an unfolded conformation, and the 86-kDa–70-kDa complex may be involved in the maintenance of this conformation.

Since many functional molecules involved in transcription or replication, like RNA polymerases, topoisomerases, etc., are phosphorylated and their activity is regulated through the phosphorylation–dephosphorylation process (Durban et al., 1981, 1983; Duceman et al., 1981), it is of interest that the 86-kDa–70-kDa protein complex is phosphorylated in vivo.

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Massive Phosphorylation Distinguishes *Xenopus laevis* Nucleoplasmin Isolated from Oocytes or Unfertilized Eggs†

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ABSTRACT: Nucleoplasmin isolated from unfertilized *Xenopus laevis* eggs possesses an in vitro chromatin assembly activity which is superior to nucleoplasmin isolated from oocytes. It is demonstrated here that the two forms of the protein differ in the amount of attached phosphate, with the egg protein possessing nearly 20 phosphate groups per protein monomer and the oocyte protein possessing less than 10 phosphate groups per monomer. A kinase preparation from unfertilized eggs is shown to be capable of modifying oocyte nucleoplasmin so that it displays the electrophoretic heterogeneity of egg nucleoplasmin. Furthermore, when the egg protein is treated with phosphatase and repurified, the chromatin assembly activity deteriorates to the level of the oocyte protein.

Nucleoplasmin is a pentameric, acidic protein which can be isolated from the oocytes or eggs of *Xenopus laevis* (Laskey et al., 1978; Mills et al., 1980). It is a major component of the oocyte nucleus, comprising 7–10% of the nuclear protein (Mills et al., 1980; Krohne & Franke, 1980). An immunologically similar protein is present in the nuclei of a number of other higher eukaryotic cell types (Krohne & Franke, 1980). Complexes containing nucleoplasmin and histones have been isolated from *Xenopus* oocyte nuclei (Kleinschmidt et al., 1985), and the protein effectively promotes the formation of nucleosomes in vitro (Laskey et al., 1977, 1978; Earnshaw et al., 1980; Sealy et al., 1986). For these reasons, nucleoplasmin is thought to play a role in the process of storage, transport, or deposition of histones onto DNA.

Nucleoplasmin isolated from oocytes is much inferior to egg nucleoplasmin in an in vitro chromatin assembly assay (Sealy et al., 1986). The two forms of the protein display different mobilities when assayed on sodium dodecyl sulfate–polyacrylamide gel electrophoresis ($NaDodSO_4$–PAGE). The egg form of the protein has a more acidic p*I* than the oocyte form when assayed by isoelectric focusing (Sealy et al., 1986). It is unlikely that the egg protein is a different gene product than the oocyte protein because radioiodinated oocyte nucleoplasmin can be converted into the egg form of the protein when it is microinjected into hormonally stimulated oocytes (M. Cotten, unpublished results), indicating that a modification of the oocyte form is sufficient for the conversion.

Thus, the most likely explanation for the differences between the oocyte and egg forms of nucleoplasmin lies in some form of posttranslational modification. There is substantial evidence in the literature of phosphorylation altering the mobility of protein on $NaDodSO_4$–PAGE (Ahmad et al., 1982; Shih et

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