Xenopus Nucleoplasmin: Egg vs. Oocyte†

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ABSTRACT: Nucleoplasmin has been purified from either oocytes or unfertilized eggs of the frog, *Xenopus laevis*. We find that the pentameric form of egg nucleoplasmin exhibits an apparent molecular mass approximately 15 000 daltons larger than its oocyte counterpart upon sodium dodecyl sulfate (SDS)–acrylamide gel electrophoresis. Egg nucleoplasmin monomers are more heterogeneous, substantially more acidic, and overall larger in apparent molecular weight than oocyte nucleoplasmin monomers when analyzed by isoelectric focusing or SDS gel electrophoresis. Protease digestions indicate that the structural differences between egg and oocyte nucleoplasmin are primarily confined to the N-terminal halves of the proteins. The structural diversity observed is accompanied by a difference in the ability of nucleoplasmin from the two sources to act as a nucleosome assembly agent in vitro. Egg nucleoplasmin efficiently promotes the formation of nucleosomes onto circular pBR322 DNA in vitro at physiological ionic strength and at physiological histone:DNA ratios, while oocyte nucleoplasmin is markedly deficient in serving as an in vitro chromatin assembly agent under all conditions which we have tested. Treatment of egg nucleoplasmin in vitro with alkaline phosphatase demonstrates that the structural diversity between egg and oocyte nucleoplasmin results primarily from extensive additional phosphorylation of the egg protein. The relevance of nucleoplasmin phosphorylation in leading to differences in the chromatin assembly activity of this protein both in vitro and in vivo is considered.

Nucleoplasmin is a major nuclear protein found in the eggs and oocytes of the frog, *Xenopus laevis* (Krohne & Franke, 1980a,b; Mills et al., 1980). Although its physiological function is unclear, the protein has served as a useful tool, both for studying the mechanism of selective transport of proteins into the nucleus (Dingwall et al., 1982) and in promoting the assembly of nucleosomes onto DNA in vitro (Laskey et al., 1977, 1978; Earnshaw et al., 1980). It is the latter function of nucleoplasmin which prompted us to isolate this protein from eggs of *Xenopus* frogs following previously published procedures (Laskey et al., 1977, 1978; Dingwall et al., 1982) with some modifications. We planned to employ purified nucleoplasmin as a chromatin assembly agent in vitro, so that the interaction between the primary component of chromatin, the nucleosome, with transcriptional control regions of DNA or other components of the transcriptional machinery might be examined in detail.

As a source for purifying substantial quantities of nucleoplasmin, *Xenopus* eggs have several drawbacks. Upon stimulation of mature *Xenopus* females with human chorionic gonadotropin, the quality and yield of eggs obtained from each frog are highly variable. Moreover, the eggs are laid over a relatively long time period (12–24 h), and they are generally unstable. Most eggs will deteriorate in 3–8 h at room temperature, necessitating frequent collections. Finally, at best, only approximately $^1/_5$th to $^1/_6$th of the total oocyte population (mature stage 6 oocytes) is laid.

We decided to circumvent these problems by isolating nucleoplasmin directly from ovaries of mature *Xenopus* frogs. We have now purified nucleoplasmin from either eggs or oocytes (all stages) of *Xenopus laevis*, and we find that there is substantial structural heterogeneity between the proteins purified from these two sources. In particular, egg nucleoplasmin is more acidic, exhibits greater heterogeneity, and migrates with a larger apparent molecular weight upon electrophoretic analysis. We also report our observations on the functional differences between egg and oocyte nucleoplasmins in serving as nucleosome assembly agents in vitro. We find that while egg nucleoplasmin, purified by our procedures, serves as an efficient chromatin assembly agent in vitro at physiological ionic strength and at physiological histone to DNA ratios, oocyte nucleoplasmin does not perform comparably under any conditions we have tested. Finally, we provide evidence that posttranslational modification by phosphorylation contributes to the structural (and perhaps functional) differences we have observed between nucleoplasmins purified from the two sources.

Experimental Procedures

Nucleoplasmin Isolation. Unfertilized eggs were obtained from mature female *Xenopus laevis* frogs after injection of human chorionic gonadotropin (1000 units/frog). Eggs were collected and dejellied as described by Laskey et al. (1977). Ovaries were dissected from mature females, rinsed several times with modified Barths saline (Laskey et al., 1977), and used intact without collagenase treatment.

Our nucleoplasmin purification procedure, starting with either ovaries or dejellied, unfertilized eggs, combines several previously published purification protocols with some modifications as indicated. A clarified homogenate from eggs or ovaries was prepared and heated to 80 °C for 10 min as detailed in Laskey et al. (1978). After centrifugation, the heat supernatant so obtained was chromatographed on a Whatman DE52 DEAE-cellulose column as described by Dingwall et al. (1982), except that the column volume was increased approximately 10-fold, the column was preequilibrated with buffer containing 100 mM NaCl and lacking β-mercapto-

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ethanol and phenylmethanesulfonyl fluoride (PMSF), and the linear gradient for elution (3 column volumes) was extended from 0.1 to 0.45 M NaCl. Fractions containing nucleoplasmin were identified by sodium dodecyl sulfate (SDS) gel electrophoresis. Nucleoplasmin generally elutes from the column at a conductivity of 32–35 $m\Omega^{-1}$. The larger DEAE column, in combination with the heat step, suffices to separate nucleoplasmin from essentially all other proteins (and nucleic acids) at this step. However, nucleoplasmin coelutes from the DEAE column with a similarly negatively charged substance which cannot be detected by Coomassie blue or silver staining, but which is detected by its ability to inhibit topoisomerase I activity during chromatin assembly assays (L. Sealy et al., unpublished results).

This inhibitory substance can be separated from nucleoplasmin by phenyl-Sepharose chromatography. DEAE column fractions containing nucleoplasmin were brought to 55% saturation with $(NH_4)_2SO_4$, applied directly to a phenyl-Sepharose column, and eluted with a linear gradient as described previously (Dingwall et al., 1982). Gradient fractions containing nucleoplasmin were identified by their absorbance at 230 nm. These fractions were pooled and then dialyzed and concentrated under negative pressure against 25 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.5, 1 mM ethylenediaminetetraacetic acid (EDTA), 150 mM NaCl, and 0.01% NP40. Purified protein is stored at −70 °C. All egg and oocyte nucleoplasmin preparations used in chromatin assembly assays were tested to ensure that no topoisomerase I inhibitory activity was present using nucleoplasmin:DNA mass ratios in excess of those employed for chromatin assembly in vitro.

Gel Electrophoresis. SDS–polyacrylamide gel electrophoresis (8%, 10%, or 15%) was performed as described by Laemmli (1970); 18% SDS gel electrophoresis was performed as described by Jackson (1978). Two-dimensional gel electrophoresis, utilizing an isoelectric focusing range of pH 4–6, was performed as described by O'Farrell (1975), except that samples were loaded from the acidic rather than basic end of the gel. Gels were stained in 40% methanol and 10% acetic acid containing 0.25% Coomassie Brilliant Blue R-250 (obtained from Bethesda Research Laboratories).

DNA samples were analyzed on 2% horizontal agarose gels of approximately 0.8-cm thickness. A buffer system of 36 mM Tris, pH 7.5, 1 mM EDTA, and 30 mM NaH_2PO_4 was employed (Shure & Vinograd, 1976), and gels were run at 2.6 V/cm for 24–26 h at room temperature. After electrophoresis, gels were stained in 1 μg/mL ethidium bromide for 30 min, destained in H_2O for 3 min, and photographed on a 300-nm UV transilluminator using an orange filter.

Monoclonal Antibody Production. A monoclonal antibody against *Xenopus* nucleoplasmin was obtained by using Balb/c mice immunized with partially or extensively purified egg nucleoplasmin following standard procedures (Galfre & Milstein, 1982). Specific details are available from the authors upon request.

Immunoblotting. Proteins analyzed on a 10% SDS–acrylamide gel as described by Laemmli (1970) were transferred electrophoretically to a nitrocellulose filter (BA85, Schleicher & Schuell) as described by Towbin et al. (1979), using the electrophoretic transfer system manufactured by Bio-rad. Gels were electroblotted at 200 mA overnight at room temperature. The filter was then blocked in phosphate-buffered saline (PBS) containing 0.05% Tween 20 (Blake et al., 1984) for 2–4 h at room temperature. The filter was exposed to anti-nucleoplasmin monoclonal antibody diluted in PBS–Tween for 2–4 h at room temperature or overnight at 4 °C. After the filter was washed 5 times for 5 min each with PBS–Tween, it was exposed to a 1:1000 dilution of alkaline phosphatase conjugated goat anti-mouse IgG (Sigma A-5153) in PBS–Tween for 1 h at room temperature. After five washes of 2 min each with PBS–Tween, the filter was developed with a solution of 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium as described by Leary et al. (1983).

Proteolysis of Nucleoplasmin. Purified egg or oocyte nucleoplasmin was digested with trypsin (Worthington, 200 units/mg) in a buffer containing 25 mM Tris, pH 8, 10 mM $CaCl_2$, and 150 mM NaCl at a concentration of 50 ng/μL at 37 °C for 90 min employing 0.075 unit of trypsin/μg of nucleoplasmin. The reactions were terminated by the addition of SDS to 0.5% and heating to 100 °C for 5 min.

Purified egg or oocyte nucleoplasmin was digested with pepsin (Sigma, 2 times crystallized, 2760 unigs/mg) in a buffer containing 0.1 M acetic acid at a concentration of 300 ng/μL at 37 °C for 30 min employing 3.5 μg of pepsin/μg of nucleoplasmin. The reaction was terminated by adding Tris, pH 8.8, and SDS to final concentrations of 0.2 M and 0.5%, respectively, followed by heating to 100 °C for 5 min.

In Vitro Chromatin Assembly. pBR322 DNA was purified from the *Escherichia coli* host, HB101, as described by Wensick et al. (1974). Hyperacetylated HTC core histones were prepared as described by Cotten and Chalkley (1984). Topoisomerase I was purchased from Promega Biotec. Non-H1-hyperacetylated HTC histones (360 ng) were mixed with an appropriate amount of nucleoplasmin for 45 min at room temperature in a final reaction volume of 48 μL containing 15 mM Tris, pH 7.5, 1 mM β-mercaptoethanol, 1 mM dithiothreitol, 1 mM sodium butyrate, 0.35 mM EDTA, 160 mM NaCl, and 0.01% NP40. Meanwhile, pBR322 DNA was relaxed with topoisomerase I (0.2–0.4 unit/μg of DNA) at room temperature for 45 min at a DNA concentration of 200 ng/μL under appropriate ionic conditions as specified by the manufacturer. Two microliters (400 ng) of DNA plus topoisomerase was then mixed with 48 μL of histone plus nucleoplasmin and the assembly reaction allowed to proceed for 2 h at room temperature. To terminate the reaction, samples were centrifuged for 5 min in an Eppendorf microfuge; supernatants were removed, and SDS was added to a final concentration of 0.2%, while pelleted material was resuspended in 20 mM Tris, pH 7.5, 160 mM NaCl, and 0.2% SDS. Both supernatant and pelleted material were incubated with 2–4 μg of proteinase K for 1–3 h at 37 °C before addition of sodium acetate to 0.3 M and 3 volumes of ethanol to precipitate the DNA. DNA samples were collected, dried, and dissolved in 36 mM Tris, pH 7.5, 1 mM EDTA, 30 mM NaH_2PO_4, 10% glycerol, and 0.025% bromophenol blue for agarose gel electrophoretic analysis.

Nucleoplasmin and histone protein concentrations were determined by analyzing appropriate samples on a 15% SDS–acrylamide gel in conjunction with a standard of calf thymus histone which had been prepared by dissolving a known amount of pure, extensively dried histone–sulfate in a known volume of H_2O. The gel was stained in fresh Coomassie Brilliant Blue R-250 obtained from Bethesda Research Laboratories. We have found Coomassie Blue stain from BRL to be more sensitive and reproducible in staining intensity than Coomassie Blue stain from other suppliers. After the gel was destained and dried between two layers of clear cellophane film, the gel was scanned on a DU8 spectrophotometer (Beckman) at 575 nm. Densitometer profiles were integrated

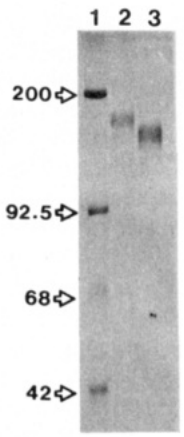

FIGURE 1: Nondissociating SDS gel electrophoresis of nucleoplasmin. Nucleoplasmin was purified from either ovaries (lane 3) or unfertilized eggs (lane 2) of *Xenopus laevis* frogs and analyzed on an 8% acrylamide–SDS gel under nondissociating conditions (see text). Coomassie Blue staining of the proteins is shown. Molecular weights markers (lane 1) were myosin (M_r 200 000), phosphorylase *a* (M_r 92 500), bovine serum albumin (M_r 68 000), and chick muscle actin (M_r 42 000).

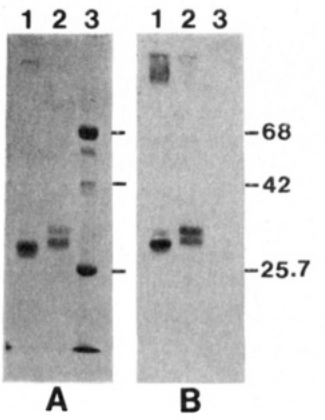

FIGURE 2: Dissociating SDS gel electrophoresis and immunoblotting of nucleoplasmin. (A) Nucleoplasmin was purified from either ovaries (lane 1) or unfertilized eggs (lane 2) of *Xenopus laevis* frogs and analyzed on a 15% acrylamide–SDS gel under dissociating conditions (see text). Molecular weight markers (lane 3) were bovine serum albumin (68K), chick muscle actin (42K), and α-chymotrypsinogen (25.7K). Coomassie Blue staining of protein is shown. (B) One-fifth of the amount of protein as described in (A) was loaded onto parallel lanes of the SDS gel and, after electrophoresis, transferred to a nitrocellulose filter. The filter was exposed to an anti-nucleoplasmin mouse monoclonal antibody followed by exposure to alkaline phosphatase conjugated goat anti-mouse IgG. Colorimetric reaction of alkaline phosphatase with the BCIP and NBT substrates is shown.

to determine protein concentrations based on the absorbance of the known standard of calf thymus histone. Samples were analyzed in duplicate and only within the linear response range of the stain. The reproducibility of this method is approximately ±5%, and values for protein concentration obtained by this method compared favorably with those obtained by quantitative amino acid analysis, which, because of the quantity of material required, was not performed routinely. DNA concentrations were determined by monitoring the absorbance at 260 nm.

Phosphatase Treatment of Nucleoplasmin. Nucleoplasmin samples were incubated with calf intestinal alkaline phosphatase (New England Nuclear) in 90 mM KCl, 1 mM dithiothreitol, 2 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 10 mM $MgCl_2$, and 100 mM Tris, pH 8.8, at 37 °C for 30 min (10 units of enzyme per microgram of nucleoplasmin) in the presence or absence of 15 mM sodium molybdate. This was followed by adding an additional 10 units of phosphatase for a further 60 min at 37 °C. The reactions were terminated by the addition of SDS to 0.5% and heating to 100 °C for 5 min.

RESULTS

Structural Heterogeneity in Xenopus Egg and Oocyte Nucleoplasmin. Xenopus nucleoplasmin was isolated from ovaries of mature female frogs or from eggs collected after injection of mature frogs with human chorionic gonadotropin. Laskey and colleagues have previously reported that nucleoplasmin isolated from *Xenopus* eggs is a pentameric protein (Earnshaw et al., 1980) composed of five subunits which run as a broad band on SDS–polyacrylamide gels with an apparent molecular weight of approximately 30 000–33 000 (Dingwall et al., 1982; Laskey et al., 1978). The pentameric form of nucleoplasmin is fairly resistant to denaturation by SDS, particularly at elevated ionic strengths (Dingwall et al., 1982). Under these conditions, heating to 100 °C in the presence of SDS and 4 M urea may be required to dissociate the pentamer into individual subunits for analysis on polyacrylamide gels.

An analysis of purified nucleoplasmin from *Xenopus* eggs or oocytes by SDS gel electrophoresis under nondissociating conditions (samples applied in 0.3 M NaCl, no heating) is presented in Figure 1. The multimeric form of nucleoplasmin from eggs (lane 2) and the multimeric form of nucleoplasmin from oocytes (lane 3) exhibit a distinct difference in apparent molecular weight. While both proteins appear as broad, somewhat diffuse bands on this 8% acrylamide gel, egg nucleoplasmin migrates with an apparent molecular weight of 165 000, whereas the protein purified by identical procedures from oocytes exhibits an apparent molecular weight of 150 000. Assuming five identical subunits in the multimeric form, this corresponds to an average subunit molecular weight of 33 000 for egg nucleoplasmin and 30 000 for oocyte nucleoplasmin.

Corresponding differences in the apparent molecular weight of the subunits are observed when purified nucleoplasmin from eggs or oocytes is analyzed by SDS gel electrophoresis under dissociating conditions (samples applied in low ionic strength, heated to 100 °C, 5 min). However, neither egg nor oocyte nucleoplasmin appears to be composed of five identical subunits. On the 10% acrylamide gel presented in Figure 2A, egg nucleoplasmin (lane 2) resolves into at least two distinct, nearly equiintense bands with apparent molecular weights of 33 500 and 31 200. In contrast, oocyte nucleoplasmin (lane 1) migrates as one major species, electrophoresing slightly faster (on average) than the most rapidly moving form of egg nucleoplasmin. The rather broad oocyte nucleoplasmin band observed in lane 1 has an average apparent molecular weight of 30 300. Also detectable in lane 1 is a very minor band (apparent M_r 32 800) which does not correspond in mobility to either of the two egg nucleoplasmin subunit forms. Staining within the major oocyte band and the two egg nucleoplasmin forms does not appear to be uniform, suggesting additional microheterogeneity may be present in the protein subunits beyond the resolution of the gel.

It is possible that the subunit heterogeneity observed when egg or oocyte nucleoplasmin is analyzed by SDS gel electrophoresis under dissociating conditions could be due to other contaminating proteins which bind to and are copurified with the multimeric form of nucleoplasmin. To address this possibility, the protein samples analyzed in Figure 2A were electrophoresed on parallel lanes of the same gel and then electrophoretically transferred to a nitrocellulose filter. The results of probing this filter with a monoclonal antibody developed in this laboratory against *Xenopus* nucleoplasmin are

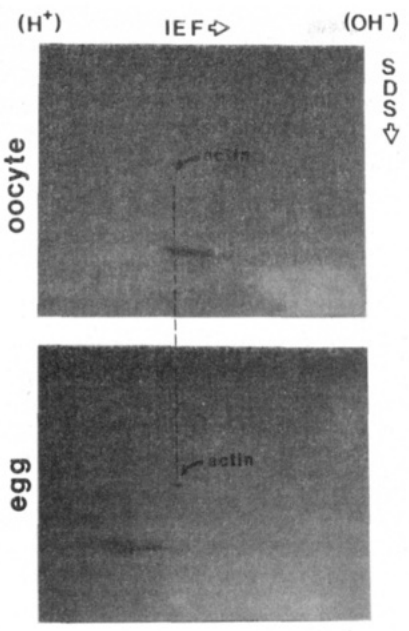

FIGURE 3: Two-dimensional electrophoresis of nucleoplasmin. Nucleoplasmin purified from *Xenopus* ovaries (top panel) or unfertilized eggs (bottom panel) was analyzed by isoelectric focusing using pH 4–6 ampholines in the first dimension followed by SDS gel electrophoresis in the second dimension. Arrows indicate the position of chick muscle actin added to each sample as an internal reference for alignment. Coomassie Blue staining of proteins is shown.

shown in Figure 2B. It is apparent that both forms of the egg nucleoplasmin subunit and the major and minor species of oocyte nucleoplasmin subunit cross-react with the monoclonal antibody. Since the protein species resolved on the 10% SDS gel in Figure 2A, lanes 1 or 2, share at least one antigenic determinant found in the multimeric form of *Xenopus* nucleoplasmin, we consider these proteins to be bona fide, although nonidentical, subunits of egg or oocyte nucleoplasmin, respectively.

The heterogeneity between the *Xenopus* egg and oocyte subunits not only results in significant shifts in the apparent molecular weight of the subunits and their composite pentameric forms but also results in substantial shifts in the isoelectric focusing points of the proteins as well. Figure 3 presents two-dimensional gel analyses of purified egg and oocyte nucleoplasmins; chicken muscle actin has been added to the purified proteins to serve as an internal reference point for alignment. It can be seen in Figure 3 that nucleoplasmin from *Xenopus* eggs is substantially more acidic than the equivalent protein isolated from oocytes. Both proteins focus as broad streaks encompassing approximately 0.7–0.9 pI unit, as has been observed previously (Krohne & Franke, 1980a,b; Mills et al., 1980). However, most of the oocyte nucleoplasmin is more basic than actin, whereas nearly all of the egg nucleoplasmin protein focuses on the acidic side of actin. Similar to their behavior on the one-dimensional SDS–acrylamide gel in Figure 2, egg nucleoplasmin resolves into two distinct species of apparently different molecular weights in the SDS second dimension, whereas the oocyte protein does not. Analysis of egg and oocyte nucleoplasmins on parallel first-dimension isoelectric focusing gels (which were then sliced and the ampholines eluted for pH measurement) indicates that the oocyte protein streak encompasses a range of 5.0–5.7 in p*I* values. The lower molecular weight species of egg nucleoplasmin span an isoelectric focusing range of p*I* 4.75 to p*I* 5.4, with the higher molecular weight forms focusing across a range of p*I* values from 4.5 to 5.0. Thus, the average shift in isoelectric focusing point between oocyte and egg nucleoplasmins is approximately 0.4 p*I* unit. A shift of this magnitude indicates that multiple additional net negative charges are present on nucleoplasmin when isolated from *Xenopus* eggs as opposed to oocytes.

At this point, we cannot exclude the possibility that the differences in molecular weight and charge between egg and oocyte nucleoplasmins are a result of selective proteolysis of the oocyte protein during its isolation, removing some highly acidic peptide(s) from the molecule. However, we consider this to be unlikely for the following reasons. (1) The differences in electrophoretic profiles of egg and oocyte nucleoplasmins are observed regardless of whether protease inhibitors are included or omitted during their respective purifications. (2) When crude extracts from eggs or oocytes are incubated at room temperature in the absence of protease inhibitors for up to 24 h, the egg or oocyte nucleoplasmin subsequently purified has the same electrophoretic behavior observed in Figures 1–3. (3) When purified egg nucleoplasmin is iodinated in vitro, added to a crude extract from oocytes, and incubated at room temperature for up to 24 h in the absence of protease inhibitors, subsequent immunoprecipitation reveals that the additional heterogeneity observed in egg nucleoplasmin has not been removed.

Localization of Structural Heterogeneity to the Protease-Resistant Core of Nucleoplasmin. We have, in fact, deliberately treated purified nucleoplasmin from both oocytes and eggs with proteases in order to ask if the heterogeneity in nucleoplasmin from these two sources could be localized to any particular domain of the protein. Laskey and colleagues (Dingwall et al., 1982) have defined various protease-resistant domains within nucleoplasmin as diagrammed in Figure 4A. Each of the five egg nucleoplasmin subunits contains a trypsin-sensitive "tail" of approximately 10000 daltons which can be removed to yield a trypsin-resistant core of approximately 23000 daltons. Pepsin also cuts within the C-terminal half of each egg nucleoplasmin subunit to yield a 16000-dalton fragment (which may be further processed to a 12000-dalton C-terminal "tail") and a pepsin-resistant core of 17000 daltons containing the N-terminus. Transport of nucleoplasmin from the cytoplasm to the nucleus requires the presence of the trypsin-sensitive tail fragments, although one 12000-dalton pepsin-resistant tail fragment per pentameric pepsin-resistant core (C_5T_1) is sufficient for nuclear accumulation (Dingwall et al., 1982). In contrast, the pentameric trypsin-resistant core is still active in nucleosome assembly in vitro (Laskey et al., 1978).

Figure 4B presents an SDS gel electrophoretic analysis of comparative proteolytic digests of egg and oocyte nucleoplasmin. When analyzed on a 15% acrylamide gel under dissociating conditions, the intact (nonproteolyzed) subunits of egg nucleoplasmin (lane 2) no longer resolve into two distinct, equiintense bands as seen on the 10% SDS gel (Figure 2). Instead, a series of closely spaced, discrete bands merging into a single broad band is observed. Intact oocyte nucleoplasmin subunits (lane 3) electrophorese as a closely spaced doublet, migrating, on average, slightly faster than the heterogeneous array of egg nucleoplasmin subunits. On the higher percentage acrylamide gel, the two resolvable subunits of intact oocyte nucleoplasmin migrate with apparent molecular weights of 27000 and 30000, whereas the subunits of egg nucleoplasmin span a molecular weight range of 28000–34000.

When egg and oocyte nucleoplasmins are digested with trypsin, the results shown in Figure 4B, lanes 4 and 5, are obtained. The trypsin-resistant monomeric core of oocyte

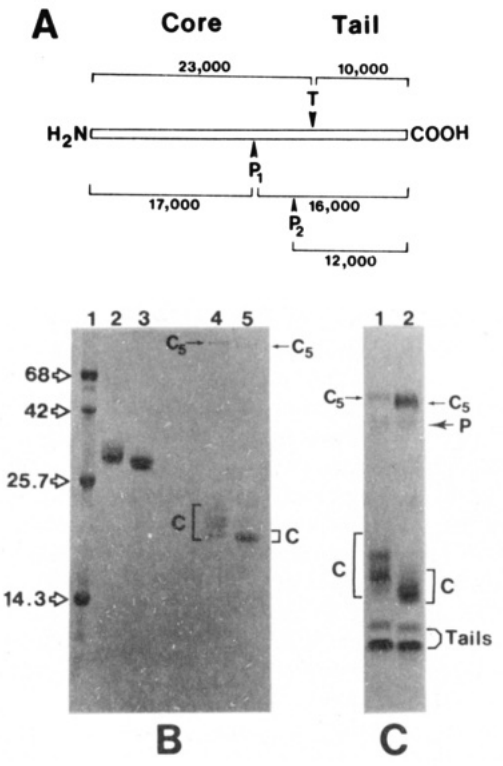

FIGURE 4: Comparative proteolysis of egg and oocyte nucleoplasmin. (A) Diagrammatic representation of cleavage sites for trypsin (T) and pepsin (P) within the egg nucleoplasmin monomer. Data taken from Dingwall et al. (1982). (B) 15% SDS gel electrophoresis of undigested egg (lane 2) and oocyte (lane 3) nucleoplasmins or trypsin-digested egg (lane 4) and oocyte (lane 5) nucleoplasmins. Molecular weight markers (lane 1) were as described in the legend to Figure 2A plus lyzozyme at M_r 14 300. Coomassie Blue staining of proteins is shown. c = core. (C) 18% SDS gel electrophoresis of egg (lane 1) or oocyte (lane 2) nucleoplasmin digested with pepsin. Molecular weight standards run in parallel with these samples, but not shown in the figure, indicate that on this particular gel system nucleoplasmin "tails" migrate with apparent molecular weights of 12 000 and 11 000. Coomassie Blue staining of proteins is shown. c = core.

nucleoplasmin migrates as a single major band of M_r 20 300. The C-terminal tail removed by trypsin is degraded to very small peptides (Dingwall et al., 1982) which are not visible on the SDS gel. The trypsin-resistant monomeric core of egg nucleoplasmin can be resolved into at least three separate and major bands (M_r's 21 200, 22 300, and 23 000) as well as several additional minor bands. The presence of multiple bands in the trypsin-treated egg nucleoplasmin is not the result of a partial digestion; further trypsin treatment with 10-fold more enzyme does not alter the electrophoretic profile of the resistant monomeric core of egg nucleoplasmin. The trypsin-resistant pentameric core of both egg and oocyte nucleoplasmin seems somewhat more resistant to dissociation than the nonproteolyzed multimer. Pentameric material resisting dissociation can be seen at the top of the gel (Figure 4B) in both lane 4 and lane 5, with the pentameric trypsinized core of oocyte nucleoplasmin migrating slightly faster than its egg counterpart. On lower percentage gels, the difference in molecular weight between egg and oocyte trypsinized pentamers is approximately 6700.

Comparison of trypsin-digested monomeric or pentameric cores of egg and oocyte nucleoplasmin strongly suggests that the structural heterogeneity between the two proteins is confined primarily to the trypsin-resistant N-terminal half of the molecule. This is substantiated by examining pepsin digests of the two nucleoplasmins on an 18% SDS–acrylamide gel as shown in Figure 4C. The initial and secondary C-terminal tail fragments produced by pepsin digestion of either egg (lane 1) or oocyte (lane 2) nucleoplasmin comigrate, while the larger pepsin-resistant fragments exhibit quite different electrophoretic mobilities. The N-terminal-containing pepsin fragments from oocyte nucleoplasmin migrate as a series of three closely spaced bands covering an apparent molecular weight range of 14 000–16 000 on this gel, plus some minor amount of more slowly migrating material. The equivalent fragments from egg nucleoplasmin resolve into multiple bands covering an apparent molecular weight range of 14 500–22 000, with a particularly predominant band at $M_{r,app}$ 19 000–20 000 and a predominant doublet at $M_{r,app}$ 16 000–17 500.

Although without exhaustive chemical analyses we cannot prove that the C-terminal 12 000 daltons of egg and oocyte nucleoplasmin are identical, it seems likely that most of the structural heterogeneity between egg and oocyte nucleoplasmin is confined to the N-terminal halves of the proteins. It is, therefore, unlikely that the structural differences which distinguish egg and oocyte nucleoplasmin are involved in nuclear transport, since these differences are not localized on that portion of the monomer (the M_r 12 000 C-terminal tail) shown to be both necessary and sufficient for rapid migration of the entire protein to the nucleus (Dingwall et al., 1982).

Functional Heterogeneity in Xenopus Egg and Oocyte Nucleoplasmin. Since the heterogeneity between egg and oocyte nucleoplasmin is not localized in that portion of the protein required for nuclear transport, we wished to test whether both egg and oocyte forms of nucleoplasmin would serve equally well as nucleosome assembly agents in vitro. We have performed assemblies at approximately physiological ionic strengths (25 mM Tris, pH 7.5, and 160 mM NaCl) utilizing pBR322 DNA and the four non-H1-hyperacetylated histones purified from HTC cells. Core histones and DNA have been employed at a mass ratio of 0.9:1 [the amount of core histones required to assemble one nucleosome per 180 base pairs (bp) of DNA]. When core histones and DNA are mixed at this mass ratio under physiological conditions, strong ionic interactions cause the mixture to precipitate (see Figure 5A,B, lanes 2 and 3) unless an assembly agent is present which can bind the histones and permit their regulated transfer onto the DNA to form nucleosomes.

The capacity of purified egg nucleoplasmin to promote nucleosome formation in vitro is shown in Figure 5A. Varying amounts of purified egg nucleoplasmin were mixed with core histones and allowed to bind for 45 min at room temperature. These mixtures were then added to pBR322 DNA which had concomitantly been relaxed by topoisomerase I for 45 min at room temperature. Chromatin assembly was allowed to proceed at room temperature in the continued presence of the topoisomerase for 2 h. Assembly mixtures were then briefly centrifuged to separate soluble from precipitated material before addition of SDS to terminate the reaction.

To visualize the negative superhelical turns introduced into the DNA by the accumulating nucleosomes (after nicking/closing action by topo I), each assembly reaction was analyzed on the high-resolution 2% agarose gel as presented in Figure 5A. The stoichiometry of superhelical turns introduced in the final deproteinized DNA per nucleosome formed is approximately 1:1 (Germond et al., 1975). Thus, the number of superhelical turns expected in pBR322 DNA (4362 bp) if it were fully assembled with one nucleosome for every 180 bp of DNA is approximately 24, which corresponds closely to the number of superhelical turns present in form I pBR322 DNA when isolated from *E. coli* [δ = −0.06, or 24–26 negative

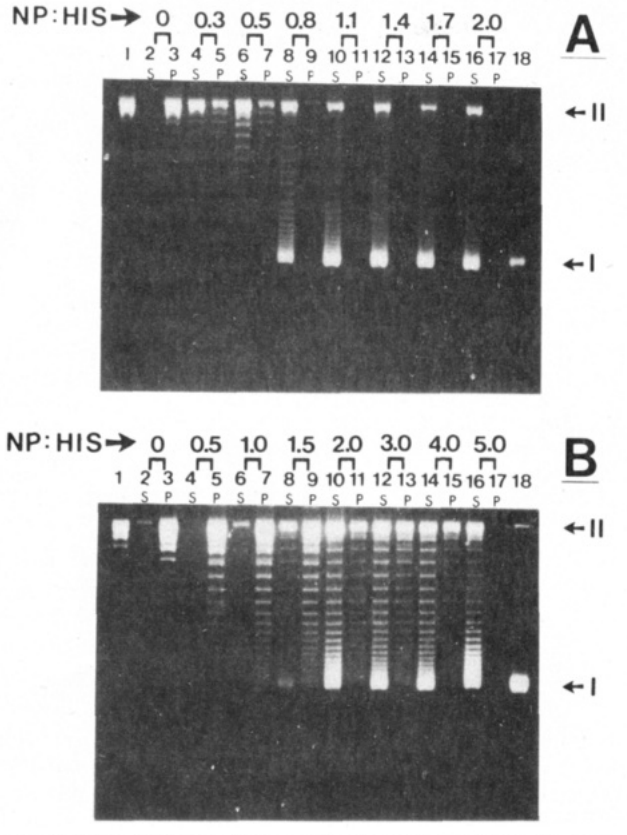

FIGURE 5: In vitro chromatin assembly capabilities of egg or oocyte nucleoplasmin. Topoisomerase I treated, relaxed pBR322 DNA (A and B, lane 1) was incubated with hyperacetylated core histones previously mixed with increasing amounts of egg nucleoplasmin (A, lanes 2–17) or oocyte nucleoplasmin (B, lanes 2–17). Mass ratios of nucleoplasmin to histone employed are shown above the lanes. After 2 h at room temperature, assembly reactions were spun at 12000*g* for 5 min, and both supernatant (S) and pelleted (P) material were analyzed on the 2% agarose gels shown in panels A and B. Supercoiled form I pBR322 DNA isolated from *E. coli* is shown in panels A and B, lane 18. On this gel system, circular pBR322 DNA containing one to two positive or negative supercoils is not resolved from nicked, circular (form II) DNA (Shure & Vinograd, 1976). Also, parallel analysis of topoisomerase I treated relaxed DNA (lane 1) in gels containing chloroquine over a range of concentrations indicate that at [chloroquine] = 0 (i.e., gels shown in this figure) the center of mass of topoisomer distribution (DNA with no supercoils present under the ionic conditions of the assembly reaction) migrates at a position of +1 supercoil (L. Karnitz, personal communication). Thus, the first topoisomer resolved from form II DNA in the agarose gels of this figure actually contains four more negative superhelical turns than the completely relaxed reference DNA (lane 1).

superhelical turns at physiological ionic strength (Sindin et al., 1980)]. Only a few negative superhelical turns are introduced into the fully relaxed pBR322 DNA initially present in the assembly reaction (lane 1) when a small amount of nucleoplasmin (0.3 μg/μg of histone) is added to the assembly mix. However, some DNA–histone complexes are now soluble (Figure 5A, lanes 4 and 5). At 0.5 μg of nucleoplasmin/μg of histone (Figure 5A, lanes 6 and 7), the DNA–histone complexes are still only partially soluble but slightly more extensively assembled, with 10–12 nucleosomes present on a few DNA molecules. When a nucleoplasmin:histone ratio of 0.8:1 is employed, all the DNA in the assembly reaction is soluble, but while some molecules have attained a full complement of nucleosomes (24–26 supercoils), incompletely assembled DNA still remains (Figure 5A, lanes 8 and 9).

Complete assembly of all pBR322 DNA molecules is achieved when 1.4 μg or more egg nucleoplasmin per microgram of histone is added to the reactions (Figure 5A, lanes 12–17). Essentially all the DNA in lanes 12, 14, and 16 migrates with a superhelical density equivalent to that of form I pBR322 DNA isolated from *E. coli* (lane 18), and thus, 24–26 supercoils, or 24–26 nucleosomes, were present on all the DNA molecules. [A small amount of DNA does remain at the position of relaxed DNA in lanes 12, 14, and 16 due to incomplete sealing of nicks by topoisomerase I when treated with SDS (Champoux, 1977) and does not indicate this DNA was devoid of nucleosomes.] To our knowledge, the in vitro assembly system we describe here utilizing hyperacetylated core histones and purified egg nucleoplasmin is the first such system of purified components to achieve complete packaging of DNA at physiological ionic strengths and at physiological histone to DNA ratios without slow and gradual additions of histone to the DNA. A histone:DNA mass ratio of 0.9:1 provides for only enough histone to assemble 24 nucleosomes on 4362 bp of DNA; essentially all the histone in the assembly reaction must be assembled into nucleosomes if all pBR322 molecules possess 24–26 nucleosomes. Other in vitro assembly agents, such as poly(glutamic acid), are less efficient at promoting nucleosome formation. Excesses of histone to DNA are required to achieve complete packaging (Stein et al., 1979; Cotten & Chalkley, 1984). In fact, previous studies with purified egg nucleoplasmin indicated that an excess of histone to DNA was required to achieve full assembly in vitro (Earnshaw et al., 1980). Moreover, the optimal histone:DNA ratio varied for each batch of purified nucleoplasmin over a wide range [histone:DNA = 2:1 to 5:1 (Earnshaw et al., 1980)].

We suspect that the previous unreliability of egg nucleoplasmin mediated nucleosome assembly in vitro and the requirement for an excess of histone were due to the presence of a contaminating substance in the otherwise purified nucleoplasmin employed in previous chromatin assembly studies. This contaminant (most probably a complex, negatively charged carbohydrate) interferes with nucleoplasmin-mediated assembly in vitro both by complexing with histones and by inhibiting topoisomerase activity (L. Sealy et al., unpublished results). The egg nucleoplasmin utilized in the assembly reactions shown in Figure 5A has been purified from this inhibitor (see Experimental Procedures) and subsequently functions as a reliable and efficient mediator of chromatin assembly in vitro.

In contrast, nucleoplasmin purified from *Xenopus* oocytes in the same manner is markedly deficient in promoting nucleosome formation in vitro when compared with its egg counterpart. Figure 5B presents an agarose gel analysis of DNA from a series of in vitro assembly reactions in which oocyte nucleoplasmin was employed as the modulator of chromatin assembly under identical ionic conditions and histone:DNA ratios as described previously. When oocyte nucleoplasmin and core histones are added to an assembly reaction in equal mass amounts (Figure 5B, lanes 6 and 7), all the DNA remains precipitated, even though lesser amounts of egg nucleoplasmin (0.8 μg) suffice to completely solubilize the DNA and promote more extensive packaging. When 1.5 μg of oocyte nucleoplasmin per microgram of histone is added to promote assembly, the majority of the DNA–protein complexes are still insoluble and incompletely assembled (Figure 5B, lane 9) while a small proportion of the soluble material is relatively well packaged (Figure 5B, lane 8). This is in sharp contrast to the capacity of 1.5 μg of egg nucleoplasmin per microgram of histone to promote assembly, in which case all DNA molecules would be fully soluble and fully assembled (see Figure 5A, lanes 12–17).

As can be seen in Figure 5B, lanes 16 and 17, a 5-fold mass excess of oocyte nucleoplasmin over histone is required to obtain soluble histone–DNA complexes, and these complexes are not yet fully packaged as evidenced by the persistance of intermediate levels of supercoiling. We have employed the purified oocyte protein at nucleoplasmin:histone mass ratios of up to 18:1 and failed to achieve further increases in the extent of assembly beyond that seen with a 3–5-fold mass excess of oocyte nucleoplasmin over histone (Figure 5B, lanes 12–17). Increasing the time of chromatin assembly to 24 h or utilizing higher histone:DNA mass ratios in assembly did not lead to fully packaged pBR322 DNA when oocyte nucleoplasmin was utilized as the assembly agent. Differences in the ability of the two nucleoplasmins to promote nucleosome assembly in vitro have been observed over a range of ionic conditions from 40 to 160 mM NaCl (data not shown). In short, we have not yet identified any experimental conditions under which oocyte nucleoplasmin can promote complete assembly of pBR322 DNA in vitro such as observed with its egg counterpart in Figure 5A.

Phosphorylation Contributes to the Structural Diversity of Xenopus Egg and Oocyte Nucleoplasmin. We have observed that egg and oocyte nucleoplasmins differ substantially in their electrophoretic mobility on SDS gels, in their isoelectric focusing behavior, and in their ability to assemble nucleosomes onto DNA in vitro. One possible explanation for these differences is that the proteins isolated from eggs or oocytes, although sharing at least one antigenic determinant, are in fact different polypeptides with different primary amino acid sequences. This would require, however, that a large store of nucleoplasmin in the mature stage 6 oocytes be degraded and a large pool of new nucleoplasmin be synthesized during the time interval between hormonal stimulation and egg laying. Ongoing studies in our laboratory indicate that the decrease in SDS gel electrophoretic mobility characteristic of egg nucleoplasmin can be observed in nucleoplasmin immunoprecipitated from stage 6 oocytes removed from frogs only several hours after human chorionic gonadotropin injection, well before the onset of egg laying (J. Kepa, unpublished observations). This is a very brief interval in which to turn over and resynthesize a large quantity of nucleoplasmin estimated at approximately 290 ng per egg (Krohne & Franke, 1980a,b; Mills et al., 1980). Therefore, we suspect that one or more posttranslational modifications, rather than de novo synthesis, is responsible for the structural differences between oocyte and egg nucleoplasmin that we have described here.

Of the many modifications known to occur to proteins postsynthetically, the modification(s) responsible for converting oocyte to egg nucleoplasmin must result in both the addition of multiple net negative charges on the egg protein and an increase in the protein's apparent molecular weight as well. Poly(ADP-ribosylation) is one such posttranslational modification that would fit these criteria. However, the absorbance at 260 nm relative to that at 230 nm for either egg or oocyte nucleoplasmin is very low (L. Sealy, unpublished results), which is contrary to that expected for a poly(ADP-ribosylated) protein. Glycosylation is another postsynthetic modification which could result in increases in both net negative charge and molecular weight. In addition to the fact that glycosylation of a nuclear protein (even if it occurred in the cytoplasm following germinal vesicle breakdown) would be highly unusual, all tests we have performed so far to detect carbohydrate moieties on egg nucleoplasmin, including binding to lectin columns and treatment with endoglycosidase H, have been negative.

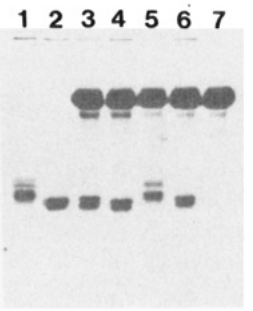

FIGURE 6: Phosphatase treatment of egg and oocyte nucleoplasmin. 15% SDS–acrylamide gel electrophoresis of purified egg (lanes 1, 3, and 5) or oocyte (lanes 2, 4, and 6) nucleoplasmin before (lanes 1 and 2) or after (lanes 3 and 4) treatment with calf intestinal alkaline phosphatase as described under Experimental Procedures. Treatment of nucleoplasmins with alkaline phosphatase in the presence of 15 mM sodium molybdate is shown in lanes 5 and 6; 30 units of alkaline phosphatase is present in lane 7. Coomassie Blue staining of proteins is shown.

It has previously been reported by Krohne and Franke (1980a,b) that oocyte nucleoplasmin is a phosphoprotein. Additional phosphorylation of nucleoplasmin during the conversion from stage 6 oocyte to laid egg would shift the isoelectric focusing range of this protein in the proper direction. Moreover, although phosphorylation of a protein does not usually affect its mobility on SDS gels, phosphorylation-induced shifts in SDS electrophoretic mobility are not unknown. Apparently, phosphorylation can at times cause a protein to undergo a conformational change that is preserved in the presence of SDS, as several examples of proteins which have been reported to exhibit phosphorylation-induced decreases in electrophoretic mobility will attest (Ahmad et al., 1982; Shih et al., 1979; Stadel et al., 1983; Wegener & Jones, 1984; Zoller et al., 1979).

To determine if "hyper"-phosphorylation of egg nucleoplasmin might be responsible for the additional heterogeneity observed in this protein when compared to its oocyte counterpart on SDS gels, we have treated egg nucleoplasmin with calf intestinal alkaline phosphatase. As shown in Figure 6, treatment with a large excess of phosphatase does remove the additional heterogeneity observed in egg nucleoplasmin, so that phosphatase-treated egg nucleoplasmin (lane 3) comigrates with untreated oocyte nucleoplasmin (lane 2) upon SDS gel electrophoretic analysis. Treatment of oocyte nucleoplasmin with an equivalent amount of alkaline phosphatase (lane 4) also results in a slight, but reproducible, increase in electrophoretic mobility, consistent with earlier reports that oocyte nucleoplasmin is phosphoprotein (Krohne & Franke, 1980a,b). However, there is no observable decrease in the heterogeneity of the oocyte protein, with both treated (lane 4) and untreated (lane 2) oocyte nucleoplasmins migrating as closely spaced doublets. This is in contrast to the large increase in electrophoretic mobility and reduction in heterogeneity when egg nucleoplasmin is treated with phosphatase, so that a series of multiple, discrete bands (untreated egg protein, lane 1) is converted to a single, closely spaced doublet (phosphatase-treated egg protein, lane 3). Incubation of the egg nucleoplasmin in lane 3 with additional phosphatase does not further increase its electrophoretic mobility to that of phosphatase-treated oocyte nucleoplasmin (M. Cotten, unpublished results), suggesting that despite the large quantity of phosphatase employed, the egg nucleoplasmin in lane 3 still contains phosphate groups which can, however, be removed from oocyte nucleoplasmin by the phosphatase treatment in vitro. Alternatively, some other structural difference may be responsible for the remaining minor difference in electrophoretic mobility between

phosphatase-treated oocyte and egg nucleoplasmins.

Finally, due to the large amount of alkaline phosphatase required to observe the shifts in electrophoretic mobility shown in Figure 6, we were concerned that some other contaminating activity (such as a protease) might be responsible for the increase in electrophoretic mobility of egg or oocyte nucleoplasmin. Accordingly, we have performed the treatments of egg or oocyte nucleoplasmin with alkaline phosphatase in the presence of 15 mM sodium molybdate, a specific inhibitor of phosphatase activity (Maggi et al., 1984). As shown in Figure 6, lanes 5 and 6, no shift in electrophoretic mobility of either egg or oocyte nucleoplasmin, respectively, is apparent upon incubation with alkaline phosphatase in the presence of a phosphatase inhibitor. Thus, we are confident that the shift in electrophoretic mobility of egg nucleoplasmin to that of oocyte nucleoplasmin shown in Figure 6 is due to the removal of phosphate groups from the egg protein in vitro.

Discussion

We have purified *Xenopus* nucleoplasmin from both unfertilized eggs and oocytes, and we find that the pentameric protein from eggs is more acidic, its subunits are more heterogeneous and of larger apparent molecular weight, and its ability to promote assembly of nucleosomes onto DNA in vitro markedly exceeds that of its oocyte counterpart. Treatment of egg nucleoplasmin with alkaline phosphatase eliminates the difference in electrophoretic behavior between egg and oocyte nucleoplasmin, so that both proteins comigrate as closely spaced doublets upon one-dimensional electrophoretic analysis and exhibit similar isoelectric focusing ranges on two-dimensional gels (L. Sealy, unpublished results). This is strong evidence that the structural diversity between egg and oocyte nucleoplasmin results from additional phosphorylation of the protein during some stage in the complex series of events which prepare a mature stage 6 oocyte for fertilization. However, it will be necessary to demonstrate conversion of oocyte nucleoplasmin to the egg form by appropriate phosphorylation to substantiate this conclusion.

We are currently able to phosphorylate exogenous purified oocyte nucleoplasmin in extracts from both *Xenopus* eggs and *Xenopus* oocytes. However, upon in vitro phosphorylation, a shift in the electrophoretic mobility of oocyte nucleoplasmin to that characteristic of egg nucleoplasmin has not been observed to date (M. Cotten, unpublished results). It is possible that the appropriate nucleoplasmin kinase is not present or active in these particular extracts and/or that the appropriate sites on oocyte nucleoplasmin are not being phosphorylated in vitro. Efforts to mimic the conversion of oocyte to egg nucleoplasmin in vitro are continuing, as well as studies aimed at defining the timing of this conversion and its possible dependence on hormonal stimulation in vivo. However, at this time, we cannot rule out the possibility that additional postsynthetic modifications may be involved in generating the structural differences between oocyte and egg nucleoplasmin we have described here.

If egg nucleoplasmin is a hyperphosphorylated form of oocyte nucleoplasmin, then is this additional phosphorylation (or other posttranslational modification) responsible for the greater capability of egg nucleoplasmin to promote nucleosome formation in vitro? As the mechanism by which *Xenopus* egg nucleoplasmin efficiently promotes nucleosome formation in vitro is not known, it is difficult to identify in what manner additional phosphorylation of the protein is required to achieve this end. Sedimentation velocity studies indicate that both egg and oocyte nucleoplasmins are capable of binding histones in vitro to a level in excess of one histone octamer per nucleoplasmin pentamer (L. Sealy, unpublished results). A priori, one might expect the additional negative charges present on egg nucleoplasmin to lead to a stronger association with positively charged histones and therefore a decreased ability to donate histones to DNA when compared with its oocyte counterpart. However, in view of our finding that the less phosphorylated oocyte protein is deficient in nucleosome assembly activity, we can conclude that if additional phosphorylation improves the assembly activity of this protein, it may do so by altering the conformation of the nucleoplasmin or the conformation in which the histones bind (or both) so that the histones are transferred more efficiently or in the proper conformation to lead to nucleosome formation. We are currently attempting to repurify egg nucleoplasmin after treatment with alkaline phosphatase in order to directly assess whether dephosphorylation is accompanied by a decline in nucleosome assembly activity in vitro.

Whether or not phosphorylation or other posttranslational modifications are responsible for modulating the chromatin assembly capabilities of nucleoplasmin in vitro, it remains unclear as to whether this abundant nuclear protein performs such a role in vivo. DNA replication does not occur in mature stage 6 oocytes while it proceeds rapidly in eggs after fertilization (Adamson & Woodland, 1974; Laskey et al., 1983). The mature oocyte germinal vesicle contains a large store of histone accumulated in preparation for the rapid rate of DNA synthesis (which exceeds that of histone) in newly fertilized eggs (Adamson & Woodland, 1974). Kleinschmidt et al. (1985) have recently provided strong evidence that oocyte nucleoplasmin is complexed in vivo with histones H2A and H2B, and possibly modified forms of histones H3 and H4, although the *s* value and electrophoretic behavior of the cross-linked complexes were used to argue that only one or two histones are bound per nucleoplasmin pentamer. Their observations and our finding of the relatively poor ability of oocyte nucleoplasmin to promote nucleosome formation in vitro would be consistent with oocyte nucleoplasmin serving as a storage factor in vivo. It is conceivable that oocyte nucleoplasmin, after being stockpiled in mature oocytes in a form conducive to its employment as a storage protein for the accumulated histone, would then be converted into its more "active" nucleosome assembly form upon egg laying, in preparation for the rapid rounds of DNA synthesis and concomitant chromatin assembly which are initiated after fertilization.

Of course, several reports have documented the capability of mature stage 6 oocytes to assemble chromatin when called upon to do so by injection of exogenous circular DNA into the oocyte germinal vesicle (Wyllie et al., 1978; Miller & Mertz, 1982; Mertz, 1982; Garguilo & Worcel, 1983; Ryoji & Worcel, 1984). However, it is not clear that the chromatin assembly process in mature oocytes occurs by the same mechanisms as the assembly process which is coupled to DNA replication in fertilized eggs. At best, the chromatin assembly process in mature oocytes may represent a slower form of that process which takes place at an accelerated rate (due to the activation of assembly factors and other changes) upon fertilization. Alternatively, if nucleoplasmin functions as an assembly factor in vivo, it may do so equally well in either its egg or its oocyte guise; the differences we have observed in its chromatin assembly behavior in vitro may simply be fortuitous and possibly a result of the requirement in our purified in vitro system for the assembly factor to interact with histones free in solution. In vivo, the histones may be complexed with other storage and transport factors, such as the N1 and N2

proteins reported to be complexed with histones H3 and H4 in the oocyte germinal vesicle (Kleinschmidt & Franke, 1982; Kleinschmidt et al., 1985).

Additional studies of the postsynthetic modifications which characterize nucleoplasmin, and the ability of the protein in each of its forms to promote nucleosome assembly in vitro, may help to distinguish between these possibilities and clarify the role of this abundant nuclear protein in vivo. For example, nucleoplasmin, or a closely antigenically related protein, has been detected by indirect immunofluorescence in a variety of somatic cells from *Xenopus* frogs and many other species as well (Krohne & Franke, 1980a,b). At present, we do not know whether nucleoplasmin in somatic cells assumes an egg- or oocyte-type form or some other state altogether. It is also not known whether nucleoplasmin from somatic sources exhibits efficient assembly activity. We are currently investigating whether the form of nucleoplasmin present in *Xenopus* eggs represents a transiently modified state or whether the protein continues in this form after fertilization as development proceeds. Whatever the function(s) of this nuclear protein in vivo, the purified form of *Xenopus* nucleoplasmin isolated from eggs should prove to be a useful tool in investigating the interactions of nucleosomes with DNA sequences or protein factors important in the control of transcriptional activity.

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REFERENCES

Adamson, E. D., & Woodland, H. R. (1974) *J. Mol. Biol. 88*, 263–285.

Ahmad, Z., DePaoli-Roach, A. A., & Roach, P. J. (1982) *J. Biol. Chem. 257*, 8348–8355.

Blake, M. S., Johnston, K. H., Russell-Jones, G. J., & Gotschlich, E. C. (1984) *Anal. Biochem. 136*, 175–179.

Champoux, J. J. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3800–3804.

Cotten, M., & Chalkley, R. (1984) *Nucleic Acids Res. 13*, 401–414.

Dingwall, C., Sharnick, S. V., & Laskey, R. A. (1982) *Cell (Cambridge, Mass.) 30*, 449–458.

Earnshaw, W. C., Honda, B. M., Laskey, R. A., & Thomas, J. O. (1980) *Cell (Cambridge, Mass.) 21*, 373–383.

Galfre, G., & Milstein, C. (1982) *Methods Enzymol. 73*, 1–46.

Garguilo, G., & Worcel, A. (1983) *J. Mol. Biol. 170*, 699–722.

Germond, J. E., Hirt, B., Oudet, P., Gross-Bellard, M., & Chambon, P. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1843–1847.

Isenberg, I. (1979) *Annu. Rev. Biochem. 48*, 159–192.

Jackson, V. (1978) *Cell (Cambridge, Mass.) 15*, 945–954.

Kleinschmidt, J. A., & Franke, W. W. (1982) *Cell (Cambridge, Mass.) 29*, 799–809.

Kleinschmidt, J. A., Fortkamp, E., Krohne, G., Zentgraf, H., & Franke, W. W. (1985) *J. Biol. Chem. 260*, 1166–1176.

Krohne, G., & Franke, W. W. (1980a) *Exp. Cell Res. 129*, 167–189.

Krohne, G., & Franke, W. W. (1980b) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1034–1038.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Laskey, R. A., Mills, A. D., & Morris, N. R. (1977) *Cell (Cambridge, Mass.) 10*, 237–243.

Laskey, R. A., Honda, B. A., Mills, A. D., & Finch, J. T. (1978) *Nature (London) 275*, 416–420.

Laskey, R. A., Harland, R. M., & Mechali, M. (1983) *Ciba Found. Symp. 98*, 25–43.

Leary, J. J., Brigati, D. J., & Ward, D. C. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 4045–4049.

Maggi, A., Schrader, W. T., & O'Malley, B. W. (1984) *J. Biol. Chem. 259*, 10956–10966.

Mertz, J. E. (1982) *Mol. Cell. Biol. 2*, 1608–1618.

Miller, T. J., & Mertz, J. E. (1982) *Mol. Cell. Biol. 2*, 1595–1607.

Mills, A. D., Laskey, R. A., Black, P., & DeRobertis, E. M. (1980) *J. Mol. Biol. 139*, 561–568.

O'Farrell, P. J. (1975) *J. Biol. Chem. 250*, 4007–4021.

Ryoji, M., & Worcel, A. (1984) *Cell (Cambridge, Mass.) 37*, 21–32.

Shih, T. Y., Weeks, M. O., Young, H. A., & Scolnick, E. M. (1979) *Virology 96*, 64–79.

Shure, M., & Vinograd, J. (1976) *Cell (Cambridge, Mass.) 8*, 15–226.

Sinden, R. R., Carlson, J. O., & Pettijohn, D. E. (1980) *Cell (Cambridge, Mass.) 21*, 773–783.

Stadel, J. M., Nambi, P., Shorr, R. G. L., Sawyer, D. R., Caron, M., & Lefkowitz, R. J. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 3173–3177.

Stein, A., Whitlock, J. P., & Bina, M. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 5000–5004.

Towbin, H., Staehelin, T., & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350–4354.

Wegener, A. D., & Jones, L. R. (1984) *J. Biol. Chem. 259*, 1834–1841.

Wensick, P. C., Finnegan, D. J., Donelson, J. E., & Hogness, D. S. (1974) *Cell (Cambridge, Mass.) 3*, 315–325.

Wyllie, A. H., Laskey, R. A., Finch, J., & Gurdon, J. B. (1978) *Dev. Biol. 64*, 178–188.

Zoller, M. J., Kerlavage, A. R., & Taylor, S. S. (1979) *J. Biol. Chem. 254*, 2408–2412.