Hyperphosphorylation of Nucleoplasmin Facilitates Xenopus Sperm Decondensation at Fertilization*

(Received for publication, January 4, 1996, and in revised form, February 7, 1996)

Gregory H. Leno‡§, Anthony D. Mills§, Anna Philpott§, and Ronald A. Laskey§

From the ‡Department of Biochemistry, University of Mississippi Medical Center, Jackson, Mississippi 39216 and the §Wellcome/CRC Institute of Cancer and Developmental Biology, Tennis Court Road, Cambridge CB2 1QR, Department of Zoology, University of Cambridge, Cambridge CB2 1QR, United Kingdom

Previous studies showed that the nuclear phosphoprotein nucleoplasmin performs the first stage of chromatin decondensation of Xenopus sperm at fertilization. It binds and removes sperm basic proteins replacing them with histones. We now show that this activity depends upon the massive hyperphosphorylation of nucleoplasmin that occurs when oocytes mature into eggs. Egg extracts or purified hyperphosphorylated egg nucleoplasmin decondense sperm chromatin and remove sperm basic proteins much faster than oocyte extracts or hyperphosphorylated oocyte nucleoplasmin. Furthermore, dephosphorylation of egg nucleoplasmin slows sperm decondensation and prevents basic protein removal from sperm chromatin. We conclude that hyperphosphorylation of nucleoplasmin is used to modulate the rapid changes in chromatin structure that accompany early development in Xenopus.

Oocyte maturation and fertilization are accompanied by major changes in chromatin structure. In Xenopus, metaphase egg cytoplasm induces chromosome condensation by the action of the cyclin-dependent kinase cd2c. In contrast, activated interphase egg extract rapidly decondenses the exceptionally compact chromatin of sperm nuclei, and it also decondenses the chromatin of introduced somatic cell nuclei (1, 2). Embryonic nuclei continue to have unstacked decondensed chromatin until the midblastula transition, which occurs at 4,000 cells. Oocyte nucleoplasmin is intermediate between the extreme states of metaphase and interphase egg cytoplasm. It will slowly induce limited chromatin decondensation of injected nuclei whereas oocyte cytoplasm does not decondense injected nuclei at all (3). We have investigated the molecular basis of these differences.

Recently, the acidic protein nucleoplasmin has been identified as the chromatin decondensation factor responsible for the rapid first stage of sperm decondensation in Xenopus laevis (4, 5). It promotes sperm decondensation by binding and removing sperm basic proteins and replacing them by histones H2A and H2B, resulting in the formation of nucleosome cores (5, 6). In this light, we have investigated the possibility that the change in chromatin decondensation activity observed between oocytes and eggs is mediated by changes in the biochemical properties of nucleoplasmin. A major change occurs to nucleoplasmin when Xenopus oocytes undergo meiosis. It becomes very highly phosphorylated, gaining 14–20 phosphates/polypeptide chain and, therefore, 70–100 phosphates/nucleoplasmin pentamer (7–9). Since nucleoplasmin is the most abundant protein in the Xenopus oocyte nucleus, occurring at 5–8 mg/ml and representing 10% of nuclear protein (10–12), the final level of phosphorylation of this single nuclear protein exceeds the amount of phosphate in oocyte DNA by a factor of 10¹⁰ (7).

We have compared the efficiency of sperm chromatin decondensation in oocyte versus egg extracts and in purified nucleoplasmin from oocytes and eggs. We observe that egg extracts and purified nucleoplasmin from eggs decondense sperm nuclei much faster than oocyte extracts or oocyte nucleoplasmin. We also observe that the remodeling of sperm chromatin, associated with decondensation, occurs in egg extracts and egg nucleoplasmin but not oocyte extracts or oocyte nucleoplasmin. Furthermore, dephosphorylation of egg nucleoplasmin slows decondensation and the remodeling of chromatin, indicating that nucleoplasmin phosphorylation is responsible for this difference in decondensation capacity between egg and oocyte nucleoplasmin.

EXPERIMENTAL PROCEDURES

Preparation of Xenopus Egg and Oocyte Extracts, Purified Nucleoplasmin, and Nuclei—Xenopus egg high speed supernatant (HSS)¹ was prepared as described by Sheehan et al. (13). Xenopus oocyte HSS was prepared according to the method of Cox and Leno (14). HSS was made 7% with respect to glycerol before freezing in liquid nitrogen. Nucleoplasmin was purified by immunoaffinity chromatography from either egg or oocyte extracts as described previously (4). Demembranated sperm nuclei were prepared from the testes of male frogs according to the method of Philpott and Leno (6).

Decondensation of Nuclei—Decondensation studies were performed essentially as described by Philpott et al. (4). Specifically, nuclei were incubated at 100 ng of DNA/μl of HSS or nucleoplasmin solution. At each time point, after gentle mixing, 2-μl aliquots were diluted with propidium iodide (final concentration, 5 μg/ml) or Hoechst 33258 (final concentration, 250 μg/ml), viewed, and photographed. Nuclei were generally incubated for between 1 and 20 min.

Polyacrylamide Gel Electrophoresis—Proteins were extracted from the chromatin and separated by Triton/acid/urea (TAU) SDS two-dimensional PAGE as described by Philpott and Leno (6) and stained with Coomassie Blue.

λ Phosphatase Treatment of Egg Nucleoplasmin—Egg nucleoplasmin was lyophilized and subsequently redissolved at ~700 ng/ml in 100 μM DTT and 1 mM MgCl₂ buffer (50 mM Tris-HCl, pH 7.8) supplemented with 2 mM MnCl₂ and 100 μg/ml acetylated bovine serum albumin. To one nucleoplasmin sample, 120 units of λ phosphatase (New England Biolabs) was added per 50 μl of the nucleoplasmin solution. To the second nucleoplasmin sample, an equal volume of λ phosphatase buffer (50 mM Tris-HCl, pH 7.8, 5 mM dithiothreitol) was added as described above. To one control sample, 5 units of λ phosphatase was added, resulting in a total of 150 units of λ phosphatase.

To whom correspondence should be addressed: Dept. of Biochemistry, University of Mississippi Medical Center, 2500 North State St., Jackson, MS 39216. Tel.: 601-984-1510; Fax: 601-984-1501; E-mail: leno@fiona.umsmed.edu.

†Recipient of a Wellcome Trust Prize Studentship. Present address: Dept. of Cell Biology, Harvard Medical School, 25 Shattuck St., Boston, MA 02115.

‡This work was supported in part by an Oak Ridge Associated Universities J. H. R. Faculty Enhancement Award (to G. H. L.), by Grant MCB-9506280 from the National Science Foundation (to G. H. L.), and by the Cancer Research Campaign (Program Grant SP1961 to R. A. L., A. D. M., and A. P.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹The abbreviations used are: HSS, high speed supernatant; TAU, Triton/acid/urea; PAGE, polyacrylamide gel electrophoresis.
plasmin phosphorylation is required for efficient sperm decondensation.

RESULTS

Egg Extracts Decondense Sperm Nuclei More Efficiently than Oocyte Extracts—We have tested the possibility that nucleoplasm phosphorylation is required for efficient sperm decondensation at fertilization. Xenopus egg extracts perform the first stage of sperm chromatin decondensation rapidly and efficiently (2, 4–6). We have compared their efficiency of decondensation with that of oocyte extracts.

Demembranated sperm nuclei were incubated at 100 ng of DNA/μl of extract in egg (13) and oocyte (14) HSSs. Samples were removed, stained with Hoechst 33258, and observed after various incubation times. After 5 min, decondensation was extensive in egg extract but minimal in oocyte extract. This difference persists but becomes less pronounced at 20 min, indicating that oocyte extracts are eventually able to decondense sperm chromatin but not at the physiological rate.

Sperm Basic Proteins Are Removed More Efficiently by Egg Extract and Egg Nucleoplasm than by Oocyte Extract and Oocyte Nucleoplasm—X. laevis sperm nuclei contain histones H3 and H4 but only trace amounts of histones H2A and H2B (5, 6, 15, 16). Instead they contain large amounts of sperm-specific basic proteins, which are presumed to be involved in chromatin compaction. Egg nucleoplasm binds and removes these basic proteins, replacing them with histones H2A and H2B to form nucleosome cores in an apparently coordinate manner (5, 6) (for review, see Ref. 17).

Fig. 1 shows that egg extract is much more efficient at removal of sperm-specific basic proteins x and y than oocyte extract. Here, as has been shown previously (5, 6), on decondensation in egg extract (Fig. 1A), removal of proteins x and y is accompanied by their replacement with histones H2A and H2B, finally giving approximately stoichiometric levels with histones H3 and H4 already present on the sperm chromatin. By contrast, when sperm nuclei are incubated in oocyte extract (Fig. 1B), proteins x and y are not removed nor are histones H2A and H2B assembled onto the DNA, consistent with the limited extent of decondensation observed with this extract.

Similar differences in decondensation (Fig. 2) and chromatin remodeling (see Fig. 4, A and B) were observed when sperm nuclei were incubated with purified egg or oocyte nucleoplasm. In egg nucleoplasm, extensive decondensation of sperm nuclei was observed within 1 min of incubation (A) while nuclei incubated in oocyte nucleoplasm for the same period of time showed little or no decondensation (C). Even after 10 min in purified oocyte nucleoplasm (D), the extent of decondensation was still less than that observed in egg nucleoplasm after 1 min (compare Fig. 2D with 2A).

Phosphatase Treatment of Egg Nucleoplasm Decreases the Efficiency of Decondensation and Sperm Chromatin Remodeling to Levels Approaching Oocyte Nucleoplasm—If the difference in chromatin decondensation activity between egg and oocyte nucleoplasm is due to nucleoplasm phosphorylation, then removing the phosphate from egg nucleoplasm should decrease its activity to approach that of oocyte nucleoplasm.

We tested this hypothesis by treating egg nucleoplasm with λ phosphatase and subsequently comparing the extent of sperm decondensation (Fig. 3) and chromatin remodeling (Fig. 4) in phosphatase-treated egg nucleoplasm with that observed in mock-treated egg nucleoplasm and oocyte nucleoplasm. As expected, decondensation in mock-treated egg nucleoplasm (Fig. 3C) was considerably more extensive than that observed with oocyte nucleoplasm (Fig. 3B). Note that at this 10-min time point, as expected, oocyte nucleoplasm has allowed limited decondensation when compared with buffer alone (Fig. 3A) (also see Fig. 2D). Phosphatase-treated egg nucleoplasm was unable to decondense sperm chromatin with the same effi-
Phosphatase treatment of egg nucleoplasmin decreases its chromatin decondensation activity. Egg nucleoplasmin was treated with λ phosphatase or mock treated as described under "Experimental Procedures." Demembranated sperm nuclei were then incubated in buffer D (BUFFER, A), oocyte nucleoplasmin (OOCYTE NPL, B), mock-treated egg nucleoplasmin (EGG NPL, C), or phosphatase-treated egg nucleoplasmin (EGG NPL + λ P'ASE, D) for 10 min. An aliquot was then removed from each sample, diluted with propidium iodide to label DNA, and photographed unfixed immediately. Scale bar, 10 μm.

Discussion

When Xenopus oocytes mature into eggs their cytoplasm acquires the ability to decondense chromatin rapidly (1, 2). This property is obviously relevant to the requirement for rapid sperm chromatin decondensation at fertilization. We have shown that different abilities of eggs and oocytes to decondense chromatin rapidly are mimicked by extracts and that this difference can be attributed to the state of the nucleoplasmin they each contain (Figs. 2 and 3). Egg nucleoplasmin decondenses sperm chromatin at the rate seen in fertilization, whereas oocyte nucleoplasmin does not (Figs. 2 and 3). Oocyte nucleoplasmin can cause very slow decondensation but far below the physiological rate. We have shown that this difference in decondensation activity can be explained by the state of phosphorylation of nucleoplasmin. Nucleoplasmin phosphorylation is correlated with its ability to decondense sperm chromatin and to remove the basic proteins x and y. Egg and oocyte nucleoplasmin differ sharply in both properties (Figs. 3 and 4). De-phosphorylation of egg nucleoplasmin by treatment with λ phosphatase decreases the rate of sperm decondensation (Fig. 3) and the extent of chromatin remodeling (Fig. 4). Dimitrov et al. (18) have shown that remodeling of Xenopus sperm chromatin is accompanied by core histone phosphorylation. While this may also contribute to subsequent decondensation events, it cannot be contributing to the decondensation shown in Figs. 2–4. The data we present in these three figures are obtained in the absence of both ATP and magnesium ions and therefore in the absence of de novo protein phosphorylation.

After phosphorylation of nucleoplasmin during oocyte maturation, the phosphate is not removed on entry into S phase. It persists until the midblastula transition, at which time the level of phosphorylation falls again (data not shown but see Ref. 19). Interestingly, this is the time that the cell cycle elongates and that the exceptionally rapid cell proliferation of the early embryo slows down. This raises the interesting possibility that nucleoplasmin phosphorylation is required to keep chromatin maximally extended to allow access of replication initiation factors at close intervals on all regions of the DNA. Such close access may be essential to allow S phases of only 20 min as seen before the midblastula transition.

The observations we describe explain several phenomena in the literature concerning Xenopus development. First, they explain why oocyte nuclei decondense injected nuclei slowly while oocyte cytoplasm does not (2, 3). Second, they explain why eggs decondense nuclei much more rapidly than oocytes (1, 2). Third, they identify a biological function of the massive phosphopeptide from egg nucleoplasmin reduces the efficiency with which it decondenses sperm chromatin.

Consistent with the observed differences in the efficiency of decondensation (Fig. 3), oocyte nucleoplasmin was also less efficient than mock-treated egg nucleoplasmin at remodeling sperm chromatin (Fig. 4). While the sperm basic proteins x and y were not removed with oocyte nucleoplasmin (Fig. 4B), most of these proteins were removed with mock-treated egg nucleoplasmin (Fig. 4A). However, phosphatase-treated egg nucleoplasmin was unable to remove x and y with the same efficiency as the mock-treated control (Fig. 4, compare C with A). Similar levels of the core histones H2A and H2B were observed in all samples (Fig. 4, A–C), indicating similar efficiencies of chromatin recovery following incubation. Purified H2A and H2B were not added to these incubations, and therefore, the levels of these core histones would not be expected to change during remodeling in mock-treated egg nucleoplasmin (Fig. 4A). As with decondensation, the extent of remodeling with phosphatase-treated egg nucleoplasmin was similar to that observed with oocyte nucleoplasmin (Fig. 4, compare C with B).
phosphorylation of nucleoplasmin, which occurs during oocyte maturation to form an egg (7–9). It will be interesting to determine if similar mechanisms are involved in fertilization of other species, particularly mammals.

Acknowledgments—We are grateful to Steve Dilworth, Colin Dingwall, and Susan Taylor for helpful discussions.

Note Added in Proof—After this work was completed corroborating data that agree well with our findings were published by Ohsumi et al. (Ohsumi, K., Shimada, A., Okumura, E., Kishimoto, T., and Kitagiri, C. (1995) Dev. Growth Differ. 37, 329–336).

REFERENCES
1. Barry, J. M., and Merriam, R. W. (1972) Exp. Cell Res. 71, 90–96
2. Lohka, M. J., and Masui, Y. (1983) Exp. Cell Res. 148, 481–491
3. Gurdon, J. B. (1976) J. Embryol. Exp. Morphol. 36, 523–540
4. Philpott, A., Leno, G. H., and Laskey, R. A. (1991) Cell 65, 569–578
5. Ohsumi, K., and Katagiri, C. (1991) Dev. Biol. 147, 110–120
6. Philpott, A., and Leno, G. H. (1992) Cell 69, 759–767
7. Laskey, R. A. (1983) Philos. Trans. R. Soc. Lond. B Biol. Sci. 302, 143–150
8. Sealy, L., Cotten, M., and Chalkley, R. (1986) Biochemistry 25, 3064–3072
9. Cotten, M., Sealy, L., and Chalkley, R. (1986) Biochemistry 25, 5063–5069
10. Mills, A. D., Laskey, R. A., Black, P., and De Robertis, E. M. (1980) J. Mol. Biol. 139, 561–568
11. Krohne, G., and Franke, W. W. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 1034–1038
12. Krohne, G., and Franke, W. W. (1980) Exp. Cell Res. 129, 167–189
13. Sheehan, M. A., Mills, A. D., Sleeman, A. M., Laskey, R. A., and Blow, J. J. (1988) J. Cell Biol. 106, 1–12
14. Cox, L. S., and Leno, G. H. (1990) J. Cell Biol. 97, 177–184
15. Mann, M., Risley, M. S., Eckhardt, R. A., and Kasinsky, H. E. (1992) J. Exp. Zool. 222, 173–186
16. Risley, M. S., and Eckhardt, R. A. (1981) Dev. Biol. 84, 79–87
17. Leno, G. H., Philpott, A., and Laskey, R. A. (1993) In John Innes Symposium: "The Chromosome" (Heslop-Harrison, J. S., and Flavell, R. B., eds) pp. 135–147, Bios Scientific, Oxford, UK
18. Dimitrov, S., Dasso, M. C., and Wolffe, A. P. (1994) J. Cell Biol. 126, 591–601
19. Burglin, T. R., Mattaj, I. W., Newmeyer, D. D., Zeller, R., and De Robertis, E. M. (1987) Genes & Dev. 1, 190–197
