HISTONES IN THE UNFERTILIZED EGG OF THE SEA URCHIN *Strongylocentrotus purpuratus*

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
LEONARD ELLIOT EVANS
1972

ABSTRACT

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By

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Conflicting evidence has existed concerning the nature of even the presence of histones in the nuclei of unfertilized sea urchin eggs. The results of this study indicate that histones are present in the unfertilized egg nucleus of the sea urchin *Strongylocentrotus purpuratus*. Electrophoretic analysis of these histones reveals a stage specific pattern which differs from that of the sperm or embryonic stages of the same species. The histones of the unfertilized egg nucleus are resolved into four distinct bands. The fastest of these, as well as the two bands of intermediate mobility, have counterparts of either similar or identical mobility in the patterns of the sperm and embryonic histones. The band of slowest mobility, however, is unique and has no counterpart in either the sperm or embryonic pattern.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Zoology

1972

ACKNOWLEDGMENTS

I would like to extend my appreciation to Dr. Hironobu Ozaki for his suggestions and assistance during the research for, and the writing of this thesis. I would also like to thank Drs. Band and Ronzio for their encouragement and help.

For their kind help throughout my stay at Michigan State University I also thank Dr. Charles S. Thornton and Mrs. Bernedette Henderson.

A special thanks is extended to Nance for her patience, moral support, and assistance throughout this study.

This work was conducted during the tenure of a National Institute of Health Graduate Traineeship (T01HD00135), and supported in part by a grant from the American Cancer Society, Michigan Division; and a Biomedical Science Support Grant of the Michigan State University awarded to Dr. Ozaki.

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The focus of this work is an investigation into the nature of the nuclear histone composition of the unfertilized egg of the sea urchin. In the following introductory comments an attempt will be made to present the rationale for undertaking such a study.

Numerous books and review articles have appeared concerning the isolation and identification of histone species and their structural and functional relationships to the nucleohistone complex (Bonner and Ts'o, 1964; Busch, 1965; Stellwagen and Cole, 1969; Georgiev, 1969; Johns, 1969; Ris and Kubai, 1970; Wilhelm, Spelsberg, and Hnilica, 1971; Elgin et al., 1971; DeLange, and Smith, 1971). Therefore only a very brief summary of the present notions regarding the role of histones as regulators of genetic activity, and as structural elements of the chromatin complex will be presented. The remainder of the introductory comments will be devoted to an analysis of the existing information concerning the nature of histones during embryonic development and gametogenesis.

In 1950, Stedman and Stedman (1950) observed that histones of salmon sperm differed markedly from those of the liver and erythrocytes of the same species. They proposed that different histone species might play an important role in the regulation of genetic transcription in differentiated tissues. In an effort to assign functional roles to various histone species, attempts have been made to correlate the appearance of a particular histone species with some aspect of genetic activity. Investigators have compared the histone composition of a wide variety of material; various tissues within an organism (Bustin and Cole, 1969; Asao, 1970; Cohen and Gotchel, 1971), the same tissue from different species (Panyim, Bilek, and Chalkley, 1971), transcriptionally inactive chromatin and chromatin actively engaged in RNA transcription (Neelin et al., 1964; Comings, 1967), and the chromatin from various stages of meiosis (Sheridan and Stern, 1967), or mitosis (Ord and Stocken, 1968; Mohberg and Rusch, 1970; Sadgopal and Bonner, 1970).

Various degrees of michroheterogeneity have been reported between histones of different tissues. Only in a few cases, however, have truly unique histones been correlated with a particular aspect of genetic activity (Neelin et al., 1964; Panyim and Chalkley, 1969a). Several investigators have demonstrated that histones are capable of non-specific repression of the genome *in vitro* (Huang and Bonner, 1962; Georgiev, Ananieva, and Kozlov, 1966; Bonner

et al., 1968a; Spelsberg and Hnilica, 1971). However, direct evidence that histones are capable of controlling transcription in a specific manner is lacking. Rather, the ubiquitous nature of the limited number of histone species as well as the results of various experiments indicate that histones might be regarded as non-specific agents capable of mediating specific control only when complexed with or directed by other chromosomal constituents (Paul and Gilmour, 1968; Bekhor, Kung and Bonner, 1969; Spelsberg, Hnilica, and Ansevin, 1971).

The structural aspects of histone DNA interactions within the nucleohistone complex are poorly understood (see review by Ris and Kubai, 1970). The nucleohistone complex contains a single DNA double helix which is associated with an approximately equal mass of histone. The various types of histone species seem to be distributed evenly along the DNA. Histones have been implicated in the folding or coiling of the DNA, however, the mechanism of this interaction remains unknown.

Histones during embryogenesis

Many investigators have attempted to determine whether or not qualitative differences in histone composition might occur during the later stages of embryonic development. Lindsay (1964); Vorobyev, Gineitis, and Vinogradova (1969); Spiegel, Spiegel, and Meltzer (1970)

have examined histones or basic proteins from the later developmental stages of various species. The electrophoretic patterns which they have presented, however, display considerable cytoplasmic contamination, and no definitive conclusions concerning the nature of the nuclear histone components can be drawn from their data.

Conflicting evidence exists concerning the nature or even the presence of histones during early development. Some cytological investigations into the nature of the chromosomal components immediately following fertilization have suggested that the nuclei of the cleavage stages of several species might contain unusual histones or lack them entirely. Other studies, however, have indicated the presence of typical histones at the same developmental stages.

Histones of adult tissue are typically demonstrated by their ability to bind alkaline fast green stain. In 1962, Bloch and Hew (1962) reported that they were unable to demonstrate the presence of alkaline fast green binding histones in the nuclei of the snail *Helix aspersa* until the onset of gastrulation. They proposed that a transition from weakly basic "cleavage histones" to more typical adult histones occurred during this early period of development. In support of this theory, Das, Kaufmann, and Gay (1964) reported that in *Drosophila melanogaster* the nuclear binding of fast green could not be detected until blastula formation. In addition, Horn (1962) reported that in the frog *Rana*

pipiens histones could not be found by the fast green procedure until gastrulation. Moore (1963) utilizing the same technique and the same species as Horn (1962), however, reported staining in the nuclei as early as the blastula stage, the earliest stage studied. Bäckström (1965) working with another amphibian species, supported the doubts raised by Horn (1962). He reported that in *Xenopus laevis* the nuclei were stained with fast green as early as the eight cell stage.

Cytochemical observations such as these are difficult to interpret since typical histones, if present, may fail to bind fast green due either to histone phosphorylation or other modification (Elgin et al., 1971) or to masking by other non-histone components.

Conflicting evidence, both cytochemical and biochemical, has been presented concerning the nature of histones during the early development of the sea urchin.

Bäckström (1965) reported that the nuclei of all developmental stages of the sea urchin *Paracentrotus lividus* were stained with fast green. He concluded that no histone transition comparable to that of the snail or fruit fly occurs in this species.

The major histone components of the prism stage of the sea urchin have been reported to be present in the 2-cell stage (Ord and Stocken, 1968), the 32-cell stage (Betinnen and Comb, 1971) and the blastula stage (Marushige

and Ozaki, 1967; Hill, Poccia and Doty, 1971; Easton and Chalkley, 1971). The relative proportions of histone species, however, have been reported to vary during development.

Collateral evidence that histones are present in the nucleus of the sea urchin during cleavage has been presented. Rinaldi and Monroy (1969) showed that polyribosome formation increased within two minutes after fertilization. These include slowly sedimenting polyribosomes, some of which are known to engage in histone synthesis (Kedes et al., 1969; Kedes and Gross, 1969a,b; Moav and Nemer, 1971). Since more than 40% of the nuclear protein produced during cleavage is acid soluble (Kedes et al., 1969), reasonable evidence has been presented to indicate that histones are synthesized and transported to the nucleus during the cleavage stages of development.

Some investigators, however, have reported that typical histones are not present in the nucleus of the cleavage stage embryos. Immers (1972) studied the ability of the chromatin to be stained by the Hale procedure and concluded that the cleavage stages of the sea urchin lacked the conventional nucleohistone complex. Orengo and Hnilica (1970) and Johnson and Hnilica (1971) were unable to demonstrate histones in the early cleavage stages of *S. purpuratus*. Asao (1969) reported similar results in the newt. In an

electrophoretic analysis of the acid soluble nuclear proteins, Johnson and Hnilica (1971) reported that typical histone patterns were not present in *S. purpuratus* prior to the 64 cell stage. Densiometric tracings of the stained gels as well as the distribution of radioactive counts indicate nine distinct protein peaks. Radioactively labeled microsomal proteins displayed the same electrophoretic mobility as these peaks, thus suggesting that considerable amounts of cytoplasmic contamination may have obscured the actual histone pattern. The potential for such contamination is high during the cleavage stages since the ratio of nuclear to cytoplasmic volume is extremely low.

Histones during gametogenesis

Spermatogenesis.--The early spermatid nucleus is usually transcriptionally active and its chromatin is diffuse. As spermatogenesis progresses, however, the chromatin becomes highly condensed and transcriptionally inert. In many species the chromosomal proteins change drastically during the course of spermatogenesis, and unusual basic proteins may be found in the nucleus of the mature spermatozoan (see review by Bloch, 1969).

Electrophoretic analysis of the histones of the sea urchin indicates that the sperm contains some histones which are similar to those of the embryo. The F1 histone of the embryo is not found in the sperm histone pattern, instead a

unique component is present. This unique component is selectively extracted with perchloric acid as is embryonic histone F1; however, it displays slower electrophoretic mobility than the latter. It is rich in both lysine and arginine, and therefore is more basic than the embryonic histone F1 (Ozaki, 1971), or histone F1 of calf thymus (Hnilica, 1967; Palau, Ruiz-Carrillo, and Subirana, 1969; Paoletti and Huang, 1969).

Oogenesis.--As in the early spermatid, the young oocyte nucleus is engaged in RNA synthesis (Gross, Malkin, and Hubbard, 1965; Piatigorsky, Ozaki, and Tyler, 1967). The extent to which the mature unfertilized egg is engaged in the synthesis of RNA has not been reliably determined. The level of incorporation of exogenously supplied RNA precursor by the mature egg is low. This low level of incorporation, however, may not necessarily reflect a low level of RNA synthesis since the egg membrane is relatively impermeable to the precursor prior to fertilization (Siekevitz, Maggio, and Catalano, 1966).

The structure of the chromatin of the young oocyte appears to undergo modification as oogenesis proceeds. Tennent and Ito (1941) reported that lampbrush chromosomes are present in the nucleoplasm of the young oocyte. At maturation, however, chromatin material is localized as a ring of Feulgen positive granules around the inside of the

nuclear membrane (Burgos, 1955; Brachet and Ficq, 1965). Agrell (1959) reported that condensed worm shaped chromosomes could be seen attached to the inside of the nuclear membrane in *P. lividus*. He reported that these chromosomes remained condensed until fertilization, at which time they became diffuse. Harris (1969) observed fairly large aggregates of finely granular Feulgen positive material at the inside of the nuclear membrane. She was uncertain, however, whether these represented whole chromosomes or only a portion of them.

Relatively few investigators have presented a biochemical analysis of the nuclear histone components of the mature unfertilized egg. Repsis (1967), Silver and Comb (1967), and Spiegel et al. (1970), claim to have isolated histones of basic proteins from the unfertilized egg of the sea urchin. Their preparations, however, were not extracted from extensively purified or washed nuclei or chromatin and hence may contain considerable amounts of cytoplasmic contamination. It is unlikely, therefore, that the electropherograms (Repsis, 1967; Spiegel et al., 1970) or chromatograms (Silver and Comb, 1967) which they have presented reflect the actual histone composition of the unfertilized egg nucleus.

Thaler, Cox, and Villee (1970) have described histones which were extracted from the purified chromatin of *Arbacia punctulata* eggs. The amino acid analysis of

their preparation indicated that the ratio of basic to acidic amino acids was 0.9 in eggs as compared to 1.8 in calf thymus. They, therefore, concluded that the acid soluble proteins of the egg nucleus were not typical histones. They reported that a large component of the unfertilized egg histone migrated with the slower lysine rich histones (F1 and F3) of calf thymus. In addition, they presented electropherograms of the histones of the sperm and embryo of the same species, and compared these to the histones of calf thymus. The comparisons derived from their results are markedly different from those of Easton and Chalkley (1972) who also conducted a similar analysis. The patterns demonstrated by Easton and Chalkley (1972) are very similar to those obtained in a study of the histones of the sperm and embryonic stages of *S. purpuratus* (Ozaki, 1971). It is, therefore, felt that the results of Thaler et al. (1970) must be viewed with caution, and that no definitive conclusion regarding the nature of unfertilized egg histones can be drawn from their data.

Hnilica and Johnson (1970) attempted to extract histones from unfertilized eggs of *S. purpuratus*. The acid soluble proteins obtained for this study were extracted from presumably purified and washed nuclei. Even so, they observed a complex electrophoretic pattern which was attributed to contaminating cytoplasmic acid soluble proteins rather than to nuclear histones. They concluded that basic

proteins similar to histones were absent from the nuclei of mature unfertilized eggs of this species.

STATEMENT OF THE PROBLEM

As noted in the previous section the results of the investigations into the nature of histones in the unfertilized egg of the sea urchin have not yielded definitive conclusions. The work of Thaler et al. (1970) indicates that histones are present in the egg nucleus of *A. punctulata*. However, since the patterns of sperm and embryonic histones which they presented are somewhat different from those presented by other investigators, the validity of the pattern which they presented for the histones of the unfertilized egg is questionable. Hnilica and Johnson (1970), on the other hand, were unable to demonstrate histones from the nuclei of the unfertilized eggs of *S. purpuratus*. Instead they found patterns which were attributed to contaminating cytoplasmic proteins and concluded that histones were absent from the nuclei of the species. An alternative hypothesis to explain the latter findings, however, might be that histones were indeed present in the nucleus, but that cytoplasmic contamination of the nuclear fraction obscured their demonstration.

The objective of the present investigation was to test the above hypothesis. It was hoped not only to

determine whether or not histones were present in the mature unfertilized sea urchin egg but also, if present, whether or not they resembled the histones of other developmental stages.

MATERIALS AND METHODS

Isolation of nuclei.--Mature eggs of the sea urchin *S. purpuratus* (Pacific Bio Marine Inc.) were obtained after the injection of 0.5 M KCl solution to induce spawning. The eggs were spawned directly into artificial sea water at 0-4°C, and all subsequent isolation procedures were carried out at this temperature unless otherwise noted. In each of five individual experiments, eggs from 8 to 10 sea urchins were pooled and washed 3 times with acidified sea water (pH 5) by hand centrifugation. Fifteen to 20 ml of packed eggs were washed 2 times with 5 volumes of a 19:1 solution of 0.53 M NaCl, 0.53 M KCl.

Nuclei were then isolated by the method of Hinegardner (1962) with some modifications. The eggs were washed 4 times with 8 volumes of 1.5 M dextrose by centrifugation at 500 g for 2 minutes. They were then cytolyzed at 10°C by the addition of 5 volumes of 0.002 M $MgCl_2$. After 3 1/4 minutes, 5 volumes of ice cold 2 M sucrose containing 0.006 M $MgCl_2$ were added and the suspension was swirled vigorously to break up the cytopyzed eggs. The lysate was centrifuged at 500 g for 10 minutes and the sediment was resuspended in 36 ml of 1.0 M sucrose, 0.004

M $MgCl_2$. Twelve ml aliquots were layered over sucrose step-gradients which were prepared according to the procedures of Hinegardner (1962) with the exception that 7 ml rather than 3.5 ml of 80% sucrose was used. After centrifugation in a Spinco SW 25.1 rotor at 56,000 g for 45 minutes, the nuclear layer was removed, diluted with an equal volume of 0.002 M $MgCl_2$, and centrifuged at 800 g for 15 minutes.

Extraction of histones.--The purified nuclei were suspended in 0.5 ml of a saline EDTA solution (0.075 M NaCl, 0.024 M EDTA, pH 8), and centrifuged in a Spinco SW 50 L rotor at 10,000 g for 10 minutes. In order to remove ribosomes and nucleoplasmic proteins, the nuclei were washed twice more with saline EDTA at the same speed, and twice with 0.01 M Tris (ph 8) by centrifugation at 17,000 g for 15 minutes. Acid soluble proteins were extracted from the nuclear pellet with 0.2 N HCl for 12 hours. After centrifugation at 10,000 g for 15 minutes the supernatant was removed and the pellet was extracted once more with 0.2 N HCl for 2 hours. The pooled supernatant farctions were used immediately for the subsequent examinations.

Sperm and embryo histones.--Total histone fractions of sperm and late blastula stage embryos were extracted with 0.2 N HCl for 30 minutes at 0°C from purified chromatin which was prepared essentially as described previously by Marushige and Ozaki (1967), and Ozaki (1971). However, a

crude embryonic nuclear pellet was prepared by homogenizing the embryos in a Sorvall omini mixer at setting 2.5 for 90 seconds. The pellet was resuspended in buffer and homogenized at setting 5 for 90 seconds to disrupt the nuclear membranes. Purification of the chromatin was then carried out as described in the above references.

Ribosomal proteins.--Eggs from 2 sea urchins were pooled and washed with acidified sea water and 19:1 saline solution as described above. Ribosomes were isolated according to a modified procedure of Wettstein, Staehelin, and Noll (1963). Four ml of packed eggs were washed 2 times in homogenization buffer (0.25 M sucrose, 0.02 M Tris, 0.15 M NH_4Cl, 0.005 M $MgCl_2$). They were resuspended in 30 ml of buffer and homogenized with 4 strokes of a loose fitting Dounce type homogenizer. The homogenate was centrifuged at 17,000 g for 20 minutes, and the upper 2/3 of the supernatant was collected. Sodium deoxycholate was added to a final concentration of 1.3%. Three 6 ml aliquots were layered over discontinuous sucrose gradients consisting of 3.5 ml of 2 M sucrose, and 3.5 ml of 1 M sucrose containing the ionic conditions of the homogenization buffer. After centrifugation in a Spinco type 40 L rotor at 105,000 g for 15 hours, the clear gelatinous pellet was resuspended in buffer (0.02 M Tris, 0.15 M NH_4Cl, 0.005 M $MgCl_2$), clarified by slow speed centrifugation, and dialyzed for 12 hours against the same buffer. The

concentration was adjusted to 20 absorbency units per ml at 260 nm. Protein was extracted from the purified ribosomes with 0.2 N HCl for 30 minutes at 0°C. After centrifugation at 10,000 g for 5 minutes, the upper 2/3 of the supernatant was collected and used immediately for the subsequent examinations.

Cytoplasmic basic proteins.--In order to compare cytoplasmic proteins with those of nuclear origin, eggs were divided by centrifugation (Harvey, 1956) to yield nucleated and enucleated egg halves. Eggs were washed with acidified sea water as described above. One-half ml of packed eggs was resuspended in 30 ml of sea water and layered over a step-gradient consisting of 9 ml of 2 parts of 1 molal sucrose to 1 part sea water, 9 ml of 3 parts of 1 molal sucrose to 1 part sea water, and 3.5 ml of 1 molal sucrose. After centrifugation in a Spinco SW 25.1 L rotor at 11,750 rpm for 10 minutes, the speed was increased to 15,500 rpm and centrifugation was continued for 10 more minutes. The layers containing the nucleated and enucleated halves were removed and diluted 1:1 with sea water. The suspensions were centrifuged at 500 g for 10 minutes and resuspended in saline EDTA. The fragments were sedimented by centrifugation in a Spinco SW 50 L rotor at 17,000 g for 20 minutes, and washed 2 times with 0.01 M Tris (pH 8) at the same speed. Basic proteins were extracted from the pellet with 0.2 N HCl at 0°C for 12 hours.

Electrophoretic analysis.--Acid soluble proteins were analyzed using 15% acrylamide gel columns (5 mm X 50 mm) containing 6 M urea (pH 4.5), and run at 4 ma/tube for 90 minutes (Bonner et al., 1968b). Proteins were stained with Amido Black 10B (Merk) and the gels were destained by diffusion. Densiometric recordings of the electropherograms were obtained using a Densiocord micro-densitometer (Photovolt Corp.).

RESULTS

The isolation method yielded nuclei substantially free from other cellular components (Figure 1).

Five separate preparations of histones from the unfertilized egg nuclei were examined by disc electrophoresis in the present study. The typical results are presented in Figure 2. The histone components of the unfertilized egg are resolved into three regions by the present electrophoretic method. The fast component of the egg has the same mobility as that of the fast component of the sperm and embryo. Histones in the intermediate region are resolved into two distinct bands. Densiometric recordings (Figure 3) of this region indicate that the peak of the faster and more prominent of these bands corresponds to the peak of the intermediate band of the embryo.

The embryonic pattern has no peak which corresponds to the slower of the intermediate bands of the egg. Histones of the intermediate region of the sperm are also resolved into two peaks. However, these peaks do not appear to correspond to those of the egg. Histones of the slow region of the electropherogram display qualitative differences. Neither the sperm nor embryo has a detectable

Figure 1. Nuclei isolated from unfertilized egg of the sea urchin *Strongylocentrotus purpuratus* according to the method of Hinegardner (1962). Phase contrast, X 700.

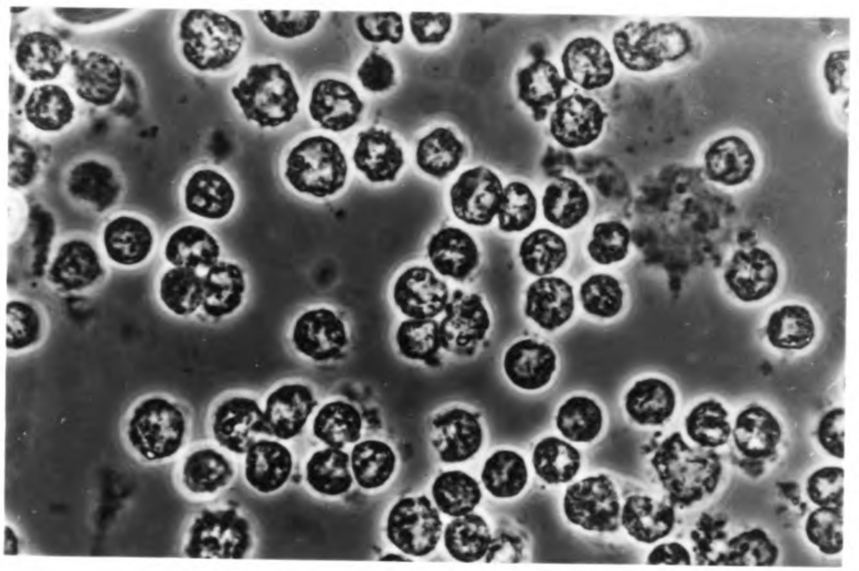

Figure 1

Figure 2. Electropherograms of the histones of (a) blastula chromatin; (b) unfertilized egg nuclei; and (c) sperm chromatin. Approximately 6 µg, 3 µg, and 10 µg of protein, respectively, were loaded on the gels. Histones are indicated by bars. The direction of migration is from the origin (O) toward the negative pole (-).

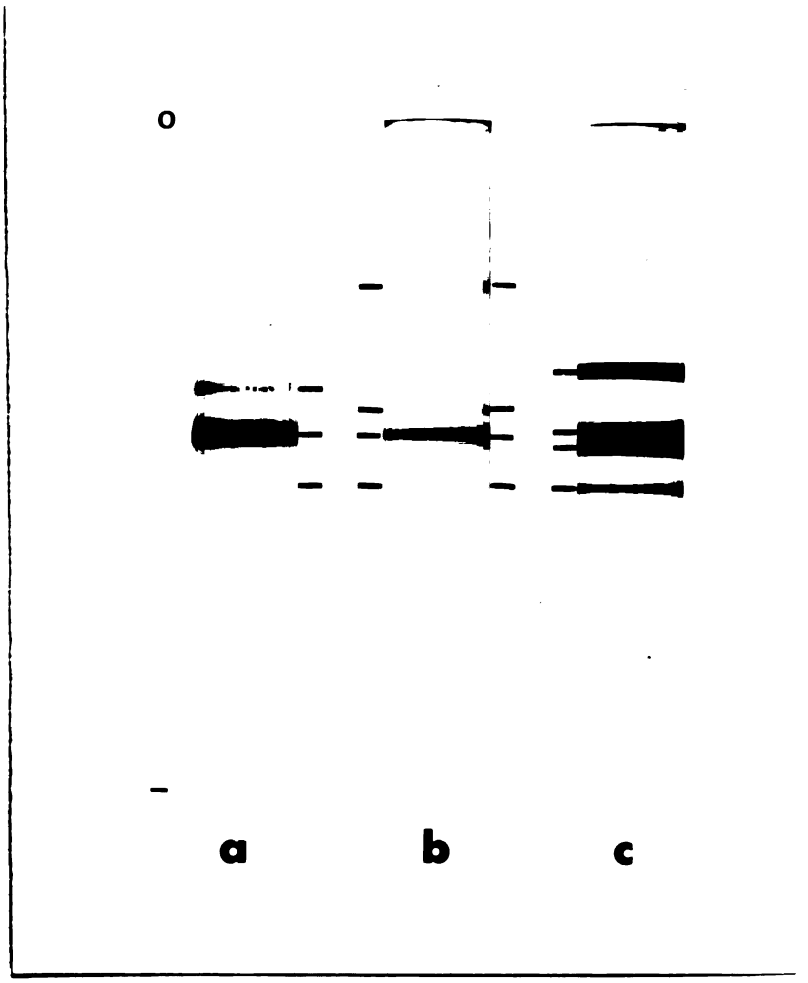

Figure 2

Figure 3. Densiometric recordings of the electropherograms of the histones of (a) unfertilized egg nuclei; and (b) blastula chromatin. The direction of migration is as indicated as in Figure 2.

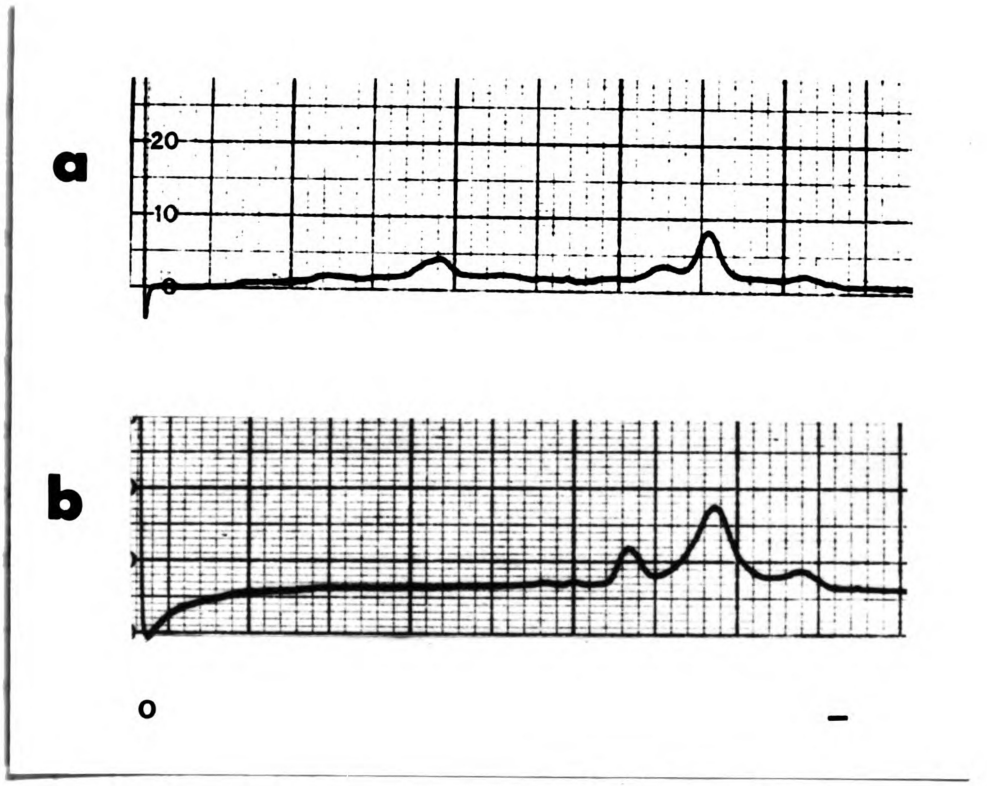

Figure 3

counterpart in the unfertilized egg pattern. The unfertilized egg pattern contains a unique component which is not present in either the sperm or embryo.

In order to demonstrate that the proteins which are designated as histones are of nuclear origin, rather than artifacts of cytoplasmic contamination, the electrophoretic patterns of the acid soluble proteins of purified ribosomes, and the 17,000 g sediment fraction of the nucleated and enucleated egg halves have been compared with the pattern of egg histones (Figure 4). The unfertilized egg pattern is markedly different from the patterns of these other basic proteins. The numerous ribosomal proteins display a wide range of electrophoretic mobilities. Some of these coincide with those of the egg histones and with the more faintly stained contaminating bands. The most intensely stained hands of the ribosomal pattern, however, have no counterparts in the egg histone pattern. Therefore, acid soluble ribosomal proteins are not a significant source of contamination in the nuclear fraction.

The electropherograms of the nucleated and enucleated halves are essentially identical. No unique bands are observed in the pattern of the nucleated halves which can be attributed to nuclear proteins. This is not surprising, however, since the ratio of cytoplasmic proteins to histones would undoubtedly obscure these bands. All of the faintly stained bands of the egg pattern have counterparts in the

Figure 4. Electropherograms of the acid soluble proteins of (a) ribosomes; (b) unfertilized egg nuclei; (c) the 17,000 g sediment of enucleated egg halves; (d) the 17,000 g sediment of nucleated egg halves. Histones are indicated by bars. The direction of migration is indicated as in Figure 2.

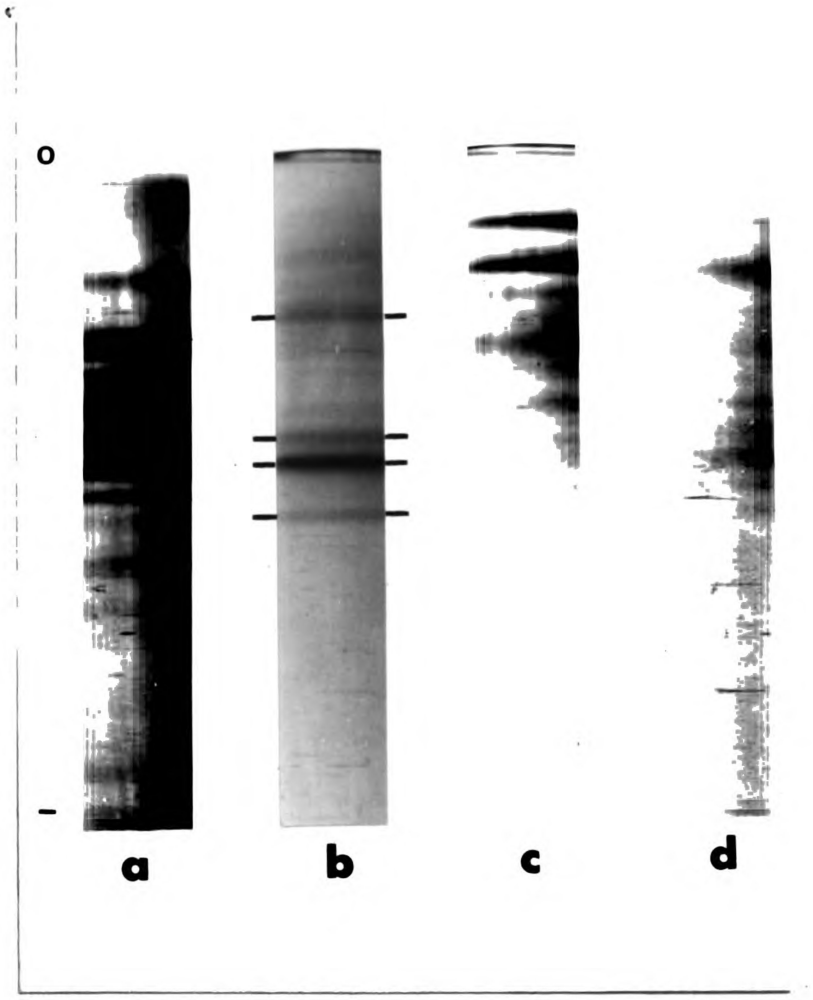

Figure 4

most intensely stained bands of the nucleated and enucleated egg patterns. These bands may therefore be attributed to cytoplasmic contamination. Though bands are found in the cytoplasmic pattern with mobilities corresponding to those of the histones, with the exception of the unique slow histone component, these bands represent minor components of the cytoplasm. Since the major cytoplasmic components are only barely visible in the egg histone pattern, the minor cytoplasmic components could not be present in detectable quantities in the histone regions of the egg pattern.

DISCUSSION

Contrary to the previous findings of Hnilica and Johnson (1970), the results of this study indicate that histones are present in the nucleus of the unfertilized egg of *S. purpuratus*. It is concluded that the four major bands present in the electrophoregram of the acid soluble proteins of the nucleus are in fact histones for the following reasons: they are acid soluble proteins extracted from nuclei whose purity has been demonstrated (Figure 1); the four band pattern is different from the patterns of acid soluble cytoplasmic protein; all of the minor bands present in the egg histone electropherogram can be attributed to a small amount of contaminating cytoplasmic protein; and at least three of the four bands display electrophoretic mobilities identical to or similar to those of histones of the sperm and embryo.

Hnilica and Johnson (1970) concluded that the acid soluble proteins which they extracted from the nuclear fraction were actually cytoplasmic contaminants. Two factors may have influenced their inability to obtain histone patterns. The ratio of the nucelar to cytoplasmic volume of the egg is approximately 1:500. Thus the potential

source of cytoplasmic contamination is greater than in most cells. In addition, the nucleus of the unfertilized egg is extremely fragile (Hinegardner, 1962; Thaler et al., 1969), and the nuclear membrane is easily disrupted. Though the isolation procedure which they used is similar to that of the present study, it would seem that either the nuclear fraction which they obtained was not as pure as in the present study, or that sufficient nuclear integrity was not maintained during their isolation steps.

The five typical classes of mammalian histones are resolved into three regions by the present electrophoretic procedure. It has been further established that the histones of the fast and slow regions are histones F2a1 and F1, respectively. The intermediate region contains the remaining three classes of histones: F3, F2b, and F2a2; however, these are not resolved into individual peaks. Recent studies of Hill et al. (1971) and Easton and Chalkley (1972) have shown that the five classes of histones are present in the nuclei of sea urchin embryos.

The apparent absence of histone F1 in the unfertilized egg pattern is consistent with the findings of Ord and Stocken (1968) who were unable to extract histone F1 from nuclei of the unfertilized egg of *P. lividus*. It is in contrast, however, to the findings of Thaler et al. (1970) who reported that a large component of the histones of the

unfertilized egg of *A. punctulata* migrated with the slower lysine rich components (F1 and F3) of calf thymus histones.

The possibility that the unique slowly migrating component of the unfertilized egg might be a polymer of histone F3 is unlikely. Polymers obtained by the oxidation of either sperm or embryo histone F3 display similar and characteristic mobility (unpublished data). The mobility of the egg component, however, is different from the characteristic mobility of the F3 polymer. The possibility that the unique component might be a complex of F3 with another non-histone component cannot be excluded. If this is the case, however, it is probable that the complex is an intrinsic component of the unfertilized egg chromatin rather than an artifact caused by the oxidation of F3 during the isolation procedure. In this study, histones were isolated at low temperatures and analyzed immediately in order to eliminate the formation of such oxidation products.

The results of this study allow the tentative conclusion to be drawn that the egg possesses a unique slowly migrating histone, as does sperm. Neither of these unique components are observed in the electropherograms of histones extracted from blastula stage embryos. The role of these unique components and their fate during the early cleavage stages is a topic requiring further investigation.

APPENDIX

APPENDIX

THE CHEMICAL COMPOSITION OF THE UNFERTILIZED EGG NUCLEUS

The chemical composition of the nuclear fraction obtained from the unfertilized eggs of *S. purpuratus* was determined.

Acid soluble proteins were determined directly from the 0.2 N HCl extract, after the sample was adjusted to 1 N NaOH, by the method of Lowry et al. (1951), using bovine serum albumin as a standard. RNA and DNA were individually determined after their separation by the method of Schmidt and Thannhauser (1945). RNA was solubilized by alkaline digestion at 37°C for 18 hr. Perchloric acid was added to a final concentration of 5%, and the suspension was centrifuged. The supernatant was removed and analyzed for RNA using the orcinol reaction with yeast RNA as a standard. The pellet was then hydrolyzed with 5% perchloric acid at 90° for 15 minutes. The suspension was centrifuged and the supernatant was analyzed for DNA using the diphenylamine method of Dische (1955) with calf thymus DNA as a standard. The phosphorus content of the RNA and DNA standards was determined with the Fisk Subbarow reagent according to the

procedure outlined by Cowgill and Pardee (1957). The amounts (mg) of DNA and RNA in the solutions were calculated by multiplying the phosphorus values (mg) by 10.1 and 10.6, respectively (Schmidt and Thannhauser, 1945). The residue after the final hot perchloric acid extraction was then dissolved in 1.0 N NaOH, and the solubilized residual protein was determined as described above.

The chemical composition of the unfertilized egg nucleus is presented in Table 1. Determination of the acid soluble proteins of the unfertilized egg according to the method of Lowry et al. (1951) yields values ten times higher than would be expected based upon the histone to DNA ratio of approximately 1:1 in chromatin of many species including the sea urchin (Bonner et al., 1968a). A rough comparison of the densiometric tracings of the electropherograms of the unfertilized egg with those of other stages which contain known amounts of protein, however, indicates that the amount of protein present in the gel is in agreement with the expected histone to DNA ratio. A similar discrepancy is found in the determination of acid soluble cytoplasmic proteins. If, however, these proteins are precipitated with trichloroacetic acid prior to the Lowry analysis, the value is reduced tenfold. It is probable that the high values in the table are the result of interference by molecules other than acid soluble proteins which had not been removed from the acid extract due to the omission

Table 1. The chemical composition of nuclei isolated from unfertilized eggs of the sea urchin *Strongylocentrotus purpuratus* according to the method of Hinegardner (1962)

	Experiment Number										Mean Value	
	45		68		75		95		107			
	μg (mass ratio)											
DNA	3.1	(1)	3.1	(1)	5.5	(1)	3.0	(1)	3.0	(1)	3.5	(1)
RNA	12.0	(3.9)	9.3	(3)	4.8	(0.9)	6.5	(2.2)	14.4	(4.8)	9.4	(2.96)
Acid Soluble Protein	39.2	(12.6)	46.3	(15)	27.0	(4.9)	28.0	(9.3)	--		35.1	(10.5)
Residual Protein	--		--		--		42.0	(14)	65.0	(21.7)	53.5	(17.9)

of the trichlorocetic acid precipitation step prior to analysis. Thus, the actual DNA to histone ratio appears to be on the order of 1:1; however, no accurate estimate can be given at the present time. Relatively large amounts of residual proteins were observed. These proteins are derived from the chromatin and nuclear membrane as well as from cytoplasmic contaminants. The amount of RNA is higher than the amount of RNA normally associated with chromatin (Bonner et al., 1968b). It is eight times lower than the amount reported by Hnilica and Johnson (1970), however. The unusually large amount of RNA observed in this study may be due to a large amount of nuclear RNA, or to orcinol reactive material other than RNA. Cytoplasmic RNA might be a contaminant. It is unlikely, however, that the RNA observed can be attributed to ribosomal contamination since no ribosomal contaminating proteins are observed in the egg histone pattern.

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