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Biochemical Aspects of Sperm Nucleus Activation by Egg Cytoplasm

Dominic L. Poccia

Department of Biology, Amherst College, Amherst, Massachusetts 01002

ABSTRACT

The sperm nucleus is highly unusual in structure and is genetically inactive. Beginning immediately after penetration of the egg at fertilization, it is transformed into a more typical nucleus. This transformation is brought about by action of the egg cytoplasm which acquires this ability late in oogenesis. Molecular details of male nuclear activation are beginning to emerge which may apply to the control of chromosome structure and gene expression in other cell types as well.

I. Introduction

The act of fertilization creates a single totipotent cell, the zygote, which in turn gives rise through a series of divisions to every other cell of the organism. Fertilization in the sea urchin involves the fusion of two highly differentiated cells, the spermatozoan and the mature oocyte or egg. It is an unequal partnership. In the laboratory the egg alone can give rise to an apparently normal organism through parthenogenesis (for example, by simple chemical stimulation). Under normal conditions, however, the sperm contributes two important elements to the zygote: a haploid set of chromosomes (the paternal gene set) and a pair of centrioles (organelles used in the organization of the apparatus used for segregating chromosomes at cell division). The remainder of the sperm provides a means for efficient motility and for penetration of and binding to the egg vestments, processes required for successful delivery of the chromosomal and centriolar payload to the egg. Virtually all else used in early embryonic development is provided by the egg. This disparity in contribution of the two gametes is suggested by the disproportionality of relative sizes of the gametes and is substantiated at the biochemical level.

As development proceeds, the embryonic nuclei (all descendents of the zygote nucleus to which the egg and sperm DNA contribute equally) become increasingly important in directing development through information sent to the cytoplasm as messenger RNA (mRNA). But the main theme of the very earliest stages of development in most organisms is the importance of maternal cytoplasm. Its effects may be mediated in two ways: 1) by direct expression of information already present in mature egg cytoplasm accumulated during differentiation of the oocyte in oogenesis, and 2) by interaction of egg cytoplasm with nuclei to alter the structure or activity of the chromosomes or chromatin. The early embryo is perhaps best viewed in terms of nucleocytoplasmic interactions, information flow occurring in both directions.

The purpose of this paper is to review information relevant to the activation of the dormant sperm nucleus following its pene-

tration of egg cytoplasm. I will first describe those features of the sea urchin sperm nucleus which distinguish it from other nuclei of the embryo or adult, then discuss macromolecular properties of egg cytoplasm which may bear upon its ability to transform the male nucleus, and finally treat some biochemical aspects of male nuclear activation. More comprehensive and technical reviews on certain of these subjects can be found in the bibliography.[1-5] References are given to illustrative papers and no attempt has been made to be comprehensive.

II. The Sperm Nucleus

A. Spermatogenesis

During the formation of the mature sperm cell (spermatogenesis), the chromatin of the precursor cell, the spermatogonium, replicates to give rise to the chromatin of the primary spermatocyte. The spermatocyte undergoes two meiotic divisions to yield four spermatids which then without division differentiate into the mature spermatozoan. The nuclei of spermatogonia look like typical somatic cell nuclei in the electron microscope. However, the nuclei of early spermatids are of an irregular shape and contain granular aggregates of chromatin displayed in no particular order against a background of more diffuse chromatin.[6] Chromatin condensation continues progressively until in the mature sperm the chromatin packing is extremely dense. Dense packing of chromatin is usually a sign of genetic inactivity. The differentiation of the spermatid (spermiogenesis) has not been studied biochemically in the sea urchin, but by analogy to other organisms, it is likely that during this period RNA synthesis ceases and sperm-specific nuclear proteins become associated with the DNA.

B. Nuclear proteins

Chromosomes of all nucleated cells except sperm contain DNA packaged with highly basic nuclear proteins called histones. There are five histone types: H2A, H2B, H3, H4 and H1. The first four (core histones) aggregate to form a structure called a nucleosome. The nucleosome consists of an octamer of two each of the core histones. DNA wraps around the outside of the core. A length of chromatin consists of an unbroken string of DNA connecting cores together. The length of DNA between cores is called linker DNA and H1 is probably associated with the linker.

Sperm cells from various organisms contain a bewildering array of nuclear proteins associated with the DNA. These range from typical histones to proteins which are not even basic.[7] In the sea urchin sperm, histones are present but three of the five (H1, H2A, and H2B) differ from the somatic or embryonic forms electrophoretically and by amino acid composition and sequence.[8-11] Sperm H3 and H4 differ little if at all from their embryonic counterparts. No multiple secondary modifications due to phosphorylation, acetylation or methylation of any of the histones has been detected, unlike histones of virtually all other cell types examined.[8] In addition levels of non-histone chromosomal proteins are extremely low.

C. Genetic inactivity

Sperm chromatin is inactive in DNA or RNA synthesis, lacks associated DNA or RNA polymerases,[12,13] and is associated with little if any RNA.[13]

D. Chromatin structure

In the electron microscope sperm chromatin shows typical nucleosomal organization,[14] and this organization is confirmed biochemically by nuclease digestion studies.[15] The typical 145 base pairs of

DNA associated with each core is separated on average by about 100 base pairs of linker DNA, making the length of one repeating unit (240–260 base pairs) the longest known for chromatin in any cell of any organism. The cores differ from embryonic cores in physical properties such as thermal stability and digestion rates or cutting sites of various nucleases.[16]

E. Summary

The sperm nucleus differs from somatic nuclei in its composition, structure, and activity. Its chromatin contains histones only found in sperm chromatin, and little if any non-histone proteins or RNA. Its chromatin is highly compact and stable and organized into long repeat units. It is inactive in DNA or RNA synthesis.

III. Maternal Storage of Macromolecules

A. RNA Synthesis During Oogenesis

As in spermatogenesis, the production of a mature egg involves transformation of an oogonium to a primary oocyte which through meiotic division gives rise to four ootids. In contrast to formation of spermatids, the divisions are unequal, producing one large cell and three small ones. In the case of the sea urchin, the large cell is the mature egg. Having completed meiosis it contains a haploid amount of nuclear DNA and is fertilized in this state. Its growth (about 2000X in volume) occurs in the primary oocyte stage, the latter part of which (vitellogenesis) involves accumulation of large amounts of yolk. During vitellogenesis large amounts of ribosomal RNA (rRNA) are synthesized and accumulate.[17] The total sequence complexity of the RNA present in the oocyte (the sum of the lengths in nucleotides of all the qualitatively *different* RNA sequences present) increases throughout development of the primary oocyte.[3]

The nuclear DNA of higher organisms falls into two classes: those nucleotide sequences which are present in essentially one copy per haploid genome, and those which are present in many copies (reiterated). Most messenger RNA's are complementary to the single copy DNA. By the time it is mature, the sea urchin oocyte contains RNA transcribed from about 6% of its single copy DNA. Much of this corresponds to structural gene sequences whose mRNA's are translated in the early embryo.[18] If all of this RNA ($30–40 \times 10^6$ nucleotides long) were mRNA it could code for as many as 30,000 different average-sized proteins.

Of the total RNA in the mature egg about 80% is ribosomal RNA.[3] Approximately 10^9 ribosomes are present per egg and maternal ribosomes account for virtually all the rRNA of early development. All other factors needed for protein synthesis such as tRNA's and various enzymes are abundant in the egg.

About 1–3% of the total mass of egg RNA is mRNA. An important class of maternal RNA is histone mRNA which is derived from the reiterated DNA sequence class. It accounts for 4–8% of the total mRNA.[3]

Not only does the mature egg contain a variety of different RNA sequences and a large amount of RNA (3.3 ng/egg), but its nucleus unlike that of the sperm is actively engaged in RNA synthesis.[19–21]

B. Precursor Pools

Mature eggs contain pools of low molecular weight molecules which are precursors in the synthesis of macromolecules. Substantial pools of ribonucleotides (RNA precursors) and deoxyribonucleotides (DNA precursors) have been measured. Total pools of deoxyribonucleotides can support embryonic DNA synthesis for several cell cycles.[22]

Amino acid pools however are only sufficient for a few minutes of protein synthe-

sis.[23] They are presumably replenished by breakdown of yolk protein which is 50–80% of the total egg protein. About 1% of total egg protein is converted per hour.

C. Protein Storage

Many enzymes and structural proteins are stored in the egg including DNA and RNA polymerases, and a variety of enzymes involved in nucleotide metabolism and protein synthesis. Of particular interest are the polymerases responsible for replicating and transcribing the DNA. Even though the number of nuclei/embryo increases exponentially during the cleavage stage of development,[24] the total activities/embryo of these enzymes remain constant.[25,26]

The presence of large amounts of deoxyribonucleotides and DNA polymerase allow the very rapid rates of DNA replication seen in the early embryo. All the new DNA must be complexed with an equal mass of histone. This is provided during development by a combination of maternally stored histone protein, translation of stored mRNA coding for histones, and translation of histone mRNA newly transcribed from the embryonic genes. The predominant type of histone found within the first few cell cycles after fertilization is of the so-called cleavage-stage (CS) variant class. These are the only variant types stored in the egg and are present in several hundred haploid equivalents/egg. They are stored therefore in great excess over the amounts required by the male or female nuclei.[9,27] Evidence for a pool of non-histone chromosomal proteins has also been presented.[28]

D. Macromolecular Responses of Egg Cytoplasm to Fertilization

The most thoroughly studied macromolecular transition occurring in the zygote following fertilization is the activation of protein synthesis.[2,3] The rate of synthesis increases about 15-fold by two hours post-fertilization.[29] This stimulation is accompanied by a 30-fold increase in the fraction of ribosomes found in polysomes but not by changes in the efficiency of translation (i.e., the rate of protein synthesis/polysomal mRNA/time).[30] Protein synthesis activation occurs even when transcription of the maternal or paternal genes is blocked with the drug actinomycin D, as well as in enucleated egg cytoplasms. Therefore it has been attributed to the recruitment of maternal mRNA from an untranslated store in the mature egg to an actively translating polyribosomal form. In the first two hours of development 90% of the polysomal mRNA is maternal whereas by gastrula stage virtually all is newly synthesized from embryonic genes.[30]

The maternal RNA is sufficient to support essentially normal development to the blastula stage as demonstrated by the ability of embryos to grow in the presence of actinomycin D. Quantitatively, the most important proteins made during cleavage stages (between fertilization and blastula) are nuclear proteins.[31] Among cleavage stage transcripts from embryonic genes, histone mRNA accounts for a substantial portion of all mRNA synthesis,[32] but by utilizing maternal histone mRNA's and stored histone and non-histone chromosomal proteins the embryo can apparently complete cleavage stages in the absence of embryonic transcription. Blocking protein synthesis, however, stops development within a single cell cycle.

An interesting question regarding the recruitment of maternal mRNA after fertilization is whether it is selective. Can a qualitatively distinct set of mRNA's be selected for translation compared to the set already being translated in the unfertilized egg? In general, it appears that most of the abundant mRNA's being translated before or after fertilization code for the same proteins.[33]

An exception is provided by the histone family. Although mRNA's coding for both

the CS variants (predominant in early cleavage chromatin) and α variants (predominant in late cleavage) are present in the unfertilized egg as demonstrated by translation in a cell-free system, in the living egg only the CS forms appear to be translated.[34] (It will be recalled that only CS variants are stored maternally.) However, after fertilization alpha variants begin to be synthesized, and so their mRNA's appear to be selectively recruited.

E. Summary

Oogenesis produces an extraordinarily large egg cell containing substantial precursor pools, enzymes of nucleic acid metabolism, histones, ribosomes and factors involved in protein synthesis, and an enormously diverse store of genetic information in the form of mRNA molecules. This maternal store of genetic information is sufficient for early development without major contribution by the embryo's genes. The egg cytoplasm can therefore control the transformation of the male nucleus following fertilization and provide for the very rapid production of chromosomes required to form a multicellular blastula embryo from a unicellular zygote.

IV. Transformation of the Sperm Nucleus by the Egg

A. Pronuclear Development

A critical event in normal early development of all organisms is the morphological and biochemical transformation of the inactive sperm nucleus. In sea urchin egg cytoplasm, the sperm nucleus becomes the male pronucleus which fuses with the egg nucleus (female pronucleus) to form the zygote nucleus. The egg pronuclear chromatin is already decondensed and active.

Decondensation and swelling of the male pronucleus in *S. purpuratus* occurs within 15–20 minutes post-fertilization proceeding from the nuclear periphery towards the core,[34,35] and during a fraction of this period the nuclear envelope is absent (see Figure 1), although the female pronucleus retains its envelope. Thus the male chromatin is rather directly exposed to egg cytoplasm. Highly condensed chromatin is characteristic of other genetically inactive nuclei and reactivation of these in foreign cytoplasms is usually accompanied by swelling and ingress of cytoplasmic proteins.[37]

The conditions which promote decondensation appear to be absent from previtellogenic or vitellogenic oocytes and only begin to appear during meiotic maturation divisions.[38] In the mature egg, however, maintenance of these conditions is not dependent on the presence of the female pronucleus, nuclear or mitochondrial RNA synthesis, or protein synthesis.[39,40] The conditions persist for a time into embryogenesis.[41]

B. Nucleic Acid Synthesis

DNA synthesis normally follows pronuclear fusion but fusion is not required. DNA synthesis in both pronuclei is initiated at 20–30 minutes post-fertilization depending on species.[42] The 30 minute lag is absent from the next few cell cycles as DNA synthesis follows immediately upon mitotic chromosome decondensation. The first mitotic condensation occurs at about 90 minutes (Figure 1). The early DNA synthetic periods in monospermic eggs may last only 5–15 minutes making them among the most rapid known in higher organisms.[42] DNA synthesis is confined to the

Fig. 1. Diagrammtic representation of various male nuclear transitions in the first cell cycle following fertilization of the sea urchin *S. purpuratus* at low degrees of polyspermy. Timing and amounts are approximate. From A. Savić, P. Richman, P. Williamson, and D. Poccia (1981). *Proc. Natl. Acad. Sci. USA* **78:** 3706–3710.

NUCLEAR MORPHOLOGY
pronuclear decondensation
mitotic condensation
DNA SYNTHESIS
replication
2.0
1.0
DNA / CELL
H1 VARIANTS
CS1
Sp1
0.2
0
H2B VARIANTS
CS2B
O,P
Sp2B
0.2
0
H2A VARIANTS
CS2A
Sp2A
0.2
0
APPROXIMATE FRACTION OF TOTAL HISTONE COMPLEMENT
NUCLEOSOME SPACING
250
200
BASE PAIRS
0
30
60
90
TIME POST - FERTILIZATION (MIN.)

nucleus. Although most DNA in the egg is mitochondrial, this DNA does not replicate in early development. It is made in oogenesis.

Nuclear RNA synthesis though difficult to detect apparently occurs in the first cell cycle.[43] The nature of the initial transcripts is not known. However, both paternal and maternal nuclear transcripts of histone mRNA can be detected as early as the two cell stage.[44]

By inference entry of DNA and RNA polymerases into male pronuclei must occur in the first cell cycle. DNA synthesis in the first cycle is largely independent of protein synthesis following fertilization, although protein synthesis seems to be required for the next round of replication.[45] Severe inhibition of nuclear or mitochondrial RNA synthesis has little effect on progression of the first cycle.[39]

C. Nuclear Proteins

Biochemical transitions must occur in the male pronucleus in the direction of those properties that distinguish embryonic nuclei from sperm nuclei as outlined in Section II. Most of these are known only by comparison of blastula or later embryonic nuclei with sperm. For several technical reasons it has been virtually impossible to study isolated male pronuclear chromatin on a biochemical level in any organism. These reasons are: 1) the high ratio of cytoplasm to nucleus in fertilized eggs which results in formidable purification problems; 2) difficulties in obtaining synchronously developing fertilized eggs in sufficient number to allow requisite quantities of chromatin for biochemical analysis to be isolated; and 3) the inability to distinguish the maternal and paternal contributions to the chromatin isolated from an egg fertilized by only one sperm. These problems have been largely circumvented in the sea urchin by use of polyspermically fertilized eggs.[36,9] Similar problems apply to analysis of the unfertilized egg nucleus or female pronucleus so biochemical information on these is limited.

Our knowledge of transitions in sperm specific nuclear proteins following fertilization has been largely based on cytochemical procedures such as staining reactions. Most studies suggest that a period occurs between the loss of sperm specific nuclear proteins and the acquisition of "typical" adult histones during which the male chromatin has proteins different from either.[46]

Using polyspermic eggs a much more detailed description is now available for the first cell cycle.[9] The first transition to take place is the complete replacement of sperm H1 by CS H1. This occurs almost immediately following fertilization and well before pronuclear decondensation is complete (Figure 1). Roughly in parallel with decondensation, a gradual loss of sperm H2B's and proportional gain of proteins O and P occur. O and P are distinguished by gel electrophoresis in the presence of a non-ionic detergent, but have virtually the same molecular weights as the sperm H2B's. O and P are as yet incompletely characterized and may represent either modified forms of the sperm H2B's or stored egg histone variants. During replication CS variants of H2A and H2B accumulate in large amounts on the chromatin, supplementing sperm H2A and O, P respectively. Several other changes are seen as well including accumulation of a form of H3 called 3″. By the time of the first chromosome condensation, male pronuclear chromatin consists of a heterogeneous group of histone variants composed of proteins the sperm nucleus brought into the egg and histone variants from the maternal store. An important point is that the chromatin remodeling that occurs in the first cell cycle is accomplished by a fairly complex set of transitions which do not occur coordinately. The discrete temporal segregation of specific transitions suggests distinct functions for each. Whether earlier transitions must occur in order for later transitions to take place is not known. The extent to which given var-

iants are secondarily modified is also unknown except for CS H1.[40] In any event male chromatin at the end of the first cycle is considerably more complex in histone composition than sperm chromatin.

All the histone transitions outlined will take place in eggs blocked in protein synthesis.[9,27] In a polyspermic egg this is taken as evidence for a minimal store of 25–50 functional haploid equivalents of each variant/egg. Independent measurements suggest an upper limit of several hundred haploid equivalents.[27]

Under conditions where DNA synthesis is blocked by aphidicolin (a DNA polymerase inhibitor) assembly of nucleosomes from the pool apparently continues.[47] In this case some of the sperm core histones must be replaced by CS variants.

The most likely candidates to be involved in decondensation of the chromatin are the H1 and H2B classes since decondensation is complete before the other transitions have progressed very far.

D. Chromatin Structure

During development of the sea urchin, sets of histone variants appear sequentially in the chromatin.[48] Once incorporated into chromatin they are stable and thus passed on to future cell generations. The elaboration of this program then must result in an increasing heterogeneity of chromatin within the organism as development progresses. The functional outcome of this increasing diversity is not known, but interesting speculations have been presented relating histone variant inheritance patterns to simple proliferative cell division and stem cell generation and maintenance.[49]

Some circumstantial evidence exists linking histone variant changes with differences in physical properties of the core histones[16] or changes in average nucleosomal repeat lengths.[50] In the first cell cycle the repeat length of the male pronuclear chromatin remains at the high level characteristic of sperm, then declines during replication to levels typical of embryonic chromatins (Figure 1). Since Sp H1/CS H1 and SpH2B/O,P transitions take place without change in spacing, they cannot at least by themselves cause the repeat length decline. H1, the noncore histone, has been a leading candidate in the literature for setting the repeat length.

The decline in repeat length coincident with replication suggests a requirement for DNA synthesis to allow adjustment of nucleosome spacing, perhaps by altering chromatin structure in such a way as to allow sliding of cores along the DNA fiber. Under conditions where DNA synthesis is blocked, the decline in repeat length is also blocked lending weight to this interpretation.[47] Since the SpH2A/CSH2A and O,P/CSH2B transitions occur in the absence of DNA synthesis they also can be ruled out as sufficient cause of the alteration in spacing.

The highest level of chromatin organization in normal cell cycles is the packing of chromatin into mitotic chromosomes. Conditions for promoting chromosome condensation have been studied in mono- and polyspermic sea urchin eggs.[39,40] These conditions are assayed either by observation of endogenous chromosomes or by chemically activating the eggs to start the maternal cell cycle and subsequently fertilizing at various times to test for the conversion of sperm chromatin directly to chromosomes (premature chromosome condensation) instead of to decondensed pronuclei. These studies show that chromosome condensing conditions can develop even in egg halves devoid of the maternal nucleus, although they are more ephemeral in enucleated eggs suggesting a role of the egg nucleus in stabilizing condensing conditions. The conditions develop independently of RNA synthesis but require protein synthesis prior to the end of replication.[39]

A biochemical transition occurring to the histone complement during mitotic or premature chromosome condensation,

also seen in other cell types, is extensive phosphorylation of histone H1 (in this case CS H1). H1 phosphorylation and chromosome condensation can however be unlinked in the egg. If protein synthesis is inhibited, CS H1 is recruited from the stored pool and becomes just as highly phosphorylated as in controls from uninhibited cultures. However chromosome condensation is blocked.Thus phosphorylation of H1 cannot drive chromosome condensation by itself. The experiment also shows that all proteins needed for CS H1 phosphorylation and its timing are already present in the unfertilized egg and suggest a role for a newly synthesized protein(s) in chromosome condensation.

V. Summary

Using the sea urchin polyspermic egg it has now become possible to describe a variety of macromolecular transitions occurring in the male pronucleus to a degree of detail never before approached. This should allow an analysis of the relationship of various aspects of the reactivation of the sperm nucleus such as protein composition, chromatin structure and genetic activity. In addition to contributing to our understanding of events of the first cell cycle of the urchin, many of the lessons learned are likely to be generally applicable to problems of chromatin structure and the activation or inactivation of replication and gene expression in a variety of cell types and organisms.

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The Cell Cycle in Early Embryonic Development

Barbara Wolfanger Belisle

Department of Biology, Georgetown University, Washington, D.C. 20057

ABSTRACT

The cell cycle in early cleavage stages of sea urchin embryos is reviewed. The embryonic cell cycle consists of S, G_2 and M (there is no G_1). Various aspects of mitotic apparatus formation, chromosome movement and the cleavage furrow are discussed. Cell cycle regulation is also reviewed, including factors involved in the control of chromosome condensation cycles and also in terms of cell cycle timing.

The eukaryotic cell cycle represents all of the events occurring in sequence from one cellular division to the next. Cells repetitively undergoing the series of events leading to division are termed cycling cells, while those cells involved in other aspects of development, growth or maintenance (for example, terminally differentiated cells) are considered to be in a non-cycling state. The mature, unfertilized sea urchin egg is one cell which is in a resting or non-cycling state. However, when the egg is fertilized and becomes metabolically activated, the first cell cycle is initiated, unleashing in the egg an awesome potential for division that can result in the production of as many as a thousand cells in 8 hours.[1] Add to this division potential the attraction of being able to fertilize an entire population of cells in such a way that all of the cells within the population divide synchronously, and the sea urchin egg becomes a useful model system for cell cycle studies. The purpose of this discussion is to review some of the events of the sea urchin cell cycle, and some of the proposed mechanisms by which the tempo of the cell cycle may be regulated. Cell cycle models derived from a study of this system must be extrapolated to other cellular systems with care. The egg is, after all, a developmental system, with a requirement for large numbers of divisions over very short time spans, with little or no interphase pause between successive cell divisions.

The basic eukaryotic cell cycle is generally subdivided into four phases designated as G_1 (Gap 1), S, G_2 (Gap 2) and M. In this scheme (Figure 1), interphase (encompassing G_1, S and G_2) is actually a dynamic state of the growing, metabolically active cell. The G_1 phase marks the interval occurring between the end of mitosis (M) and the onset of the DNA synthetic phase (S). It