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## Remodelling the paternal chromatin at fertilization in mammals

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### Abstract

At fertilization, the highly condensed and transcriptionally inert chromatin of the spermatozoa becomes remodelled into the decondensed and transcriptionally competent chromatin of the male pronucleus. The chromatin initially becomes dispersed and then transiently recondenses into a small mass upon entry into the ooplasm. This morphological change is coincident with and likely dependent on the replacement of the sperm-specific protamines by oocyte-supplied histones and the organization of the chromatin into nucleosomes. The chromatin then extensively decondenses within the male pronucleus and acquires many of the proteins that are associated with the maternal chromatin. Nonetheless, the paternal chromatin manifests distinct characteristics, including transient hyperacetylation of histone H4, increased transcription of endogenous and microinjected genes, and replication-independent demethylation of DNA. Sperm chromatin remodelling is controlled by an oocyte activity that appears during meiotic maturation and disappears approximately 3 h after activation (release from metaphase II arrest), and which requires factors associated with the germinal vesicle of the oocyte. The molecular components of this activity remain largely unknown. In frogs, nucleoplasmin is required to assemble histones H2A and H2B onto the paternal chromatin. Evidence is presented that related proteins may perform similar functions in mammals. Identifying the mechanisms that underlie sperm chromatin remodelling at fertilization may be relevant for understanding reprogramming of somatic cell nuclei after transfer into oocytes.

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Fertilization achieves two objectives: it re-initiates the cell cycle of the dormant egg, and it produces an embryo that contains one functional copy each of the maternal and paternal genomes. The second objective is ensured through a variety of mechanisms, including the block to polyspermy, the completion of the second meiotic division in the oocyte, and the biochemical remodelling of the sperm chromatin to form the male pronucleus. Remodelling of the paternal chromatin at fertilization is necessary because although the paternal genome is required for embryonic development, the DNA of the mature spermatozoa is compacted and transcriptionally inactive. The process through which this remodelling occurs is reviewed here.

Biparental androgenetic and gynogenetic mammalian embryos produced by pronuclear transplantation arrest development at mid-gestation (McGrath and Solter, 1984; Surani *et al.*, 1984). These well-known experiments of McGrath and Solter (1984) and Surani *et al.*

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(1984) established that normal mammalian development requires transcription from both parental genomes. In mice, transcription by RNA polymerase II is first observed in the late one-cell embryo, initially from the paternal pronucleus (Bouniol *et al.*, 1995; Aoki *et al.*, 1997), and is followed by the major activation of the embryonic genome at the two-cell stage (Flach *et al.*, 1982). Likewise, transcripts of Y-linked genes *Zfy* and *Sry* are present in one-cell human zygotes (Ao *et al.*, 1994), and expression from paternal copies of autosomal genes is observed at the time of activation of the human embryonic genome, the four-cell stage (Taylor *et al.*, 1997). Thus, in the embryo, the paternal genome is transcribed from the earliest stages of embryogenesis and is necessary for successful development.

## Sperm chromatin before fertilization

The chromatin of mature mammalian spermatozoa differs markedly in composition and structure from somatic chromatin. The DNA is not associated with histones or arranged in nucleosomes. Instead, it is complexed with sperm-specific basic proteins in a crystalline-like structure that, being sixfold more compact than metaphase chromosomes, is the most highly condensed chromatin in eukaryotes. The predominant chromatin-associated protein is protamine P1: a small (50 amino acid), highly basic protein possessing an arginine-rich central domain (reviewed in Wouters-Tyrou *et al.*, 1998). Mice and humans are exceptional among mammals in that the spermatozoa also contain a second protamine, P2. The Balhorn model posits that the arginine-rich core of the protamine lies lengthwise in the minor groove of the DNA double helix, filling one helical turn (Balhorn, 1982). This neutralizes the negative charge of the DNA phosphate backbone, allowing close alignment of adjacent DNA strands to form a sheet-like arrangement. Inter-protein disulphide bridges anchor the protamines.

The replacement of histones by protamines is a multistep process during the final stages of spermatogenesis (reviewed in Wouters-Tyrou *et al.*, 1998). DNA is complexed with histones and organized in nucleosomes throughout meiosis to the haploid spermatid stage. As a prelude to removal of the histones, the stable nucleosome structure is relaxed by processes linked with acetylation of histone H4 (Meistrich *et al.*, 1992) and ubiquitination of H2B in mice and H3 in rats (reviewed in Jason *et al.*, 2002). During spermatid elongation and nuclear condensation, histones are replaced by transition proteins TP1–4, which in turn are replaced by pro-protamines, one of which is cleaved to produce mature P2 (Yelick *et al.*, 1987). Haplo-insufficiency of the genes encoding P1 or P2 results in aberrant sperm chromatin condensation and structure, and decreased fertility (Cho *et al.*, 2001). Although protamines P1 and P2 are the major nucleoproteins of mature spermatozoa, human sperm chromatin may contain up to 15% histones (Gatewood *et al.*, 1987). Moreover, by fluorescent *in situ* hybridization, histone H2B co-localizes with telomeric DNA in spermatozoa (Zalensky *et al.*, 1997), and the telomere-binding protein complex isolated from human spermatozoa contains a variant of H2B (Gineitis *et al.*, 2000). This evidence indicates that even in mature spermatozoa, some chromatin remains arranged into nucleosomes. Nonetheless, chromatin of mature spermatozoa is transcriptionally inert, presumably as a result of its unique structure and composition.

## Morphological and molecular remodelling of the sperm chromatin at fertilization

At fertilization, the oocyte faces the challenge of remodelling the condensed sperm chromatin into an accessible, transcriptionally competent form. This process is described below and in Fig. 1. Upon entry into the oocyte, the sperm nucleus undergoes marked morphological changes. This remodelling follows a characteristic tri-phasic pattern (Wright and Longo, 1988; Adenot *et al.*, 1991). In the first phase, during the period that the oocyte is completing anaphase II, the paternal chromatin disperses. Although the change in volume is difficult to calculate, the area occupied by the chromatin as seen using the light microscope increases about threefold. While the oocyte chromosomes are completing telophase II, the chromatin transiently recondenses into a small mass, the area it occupies decreasing by about one-half. In the final phase, the chromatin extensively decondenses within the male pronucleus and the area it occupies increases about 10-fold, in synchrony with the oocyte chromatin that decondenses in the separate female pronucleus.

Morphological remodelling is accompanied by the replacement of protamines by histones. Inactivation of sulphhydryl-dependent enzymes by iodoacetamide or depletion of oocyte glutathione prevents sperm decondensation, implying that the reduction of inter-protamine sulphhydryl bonds is a necessary step for their removal (reviewed by Perreault, 1992). A variety of techniques, including autoradiographic detection of tritiated chromatin-associated proteins and immunostaining using antibodies against sperm basic proteins, have established that protamines are removed from sperm chromatin by the end of oocyte anaphase II (Ecklund and Levine, 1975; Kopečný and Pavlok, 1975; Rodman *et al.*, 1981). In a similar way, histones become immunologically detectable before the first round of embryonic DNA replication begins (Nonchev and Tsanev, 1990; Adenot *et al.*, 1997). Nonetheless, the precise timing of histone assembly on to the paternal chromatin at fertilization has not been established. However, in an experimental study in which oocytes were inseminated at the beginning of maturation, histone assembly was closely correlated with the transient recondensation observed morphologically (McLay and Clarke, 1997). It seems likely that this same relationship holds during normal fertilization and that the morphological recondensation reflects histone assembly onto the chromatin.

After histone assembly and its organization into nucleosomes, the paternal chromatin becomes decondensed within the male pronucleus. During this time, additional proteins must be imported from the ooplasm into the pronucleus (as indicated by its continued enlargement), where they become directly or indirectly associated with the chromatin. For example, kinetochore proteins are assembled on to male chromatin during pronuclear development. Using the CREST antibody, which recognizes centromeric proteins CENP-A and CENP-B, Schatten *et al.* (1988) showed that mature mouse spermatozoa did not contain immunodetectable kinetochores. Shortly after fertilization, while the male pronucleus was still growing, CENP-A–B appeared on the paternal chromatin. Likewise, the proteins required for DNA replication must also be assembled onto the paternal chromatin. Priming the genome for DNA replication has been shown to involve several steps in somatic cells, for example, assembly during late telophase of minichromosome maintenance proteins MCM2–

7 on DNA to form pre-replication complexes (Dimitrova *et al.*, 2002). It seems reasonable to suppose that during chromatin remodelling at fertilization, MCM proteins would also enter the male pronucleus to prepare the paternal chromatin for the S-phase.

Surprisingly, although the male and female pronuclei reside in the same ooplasm, several differences become established between them during the first cell cycle. During the early portion of the first cell cycle, histone H4 in the paternal pronucleus is hyperacetylated compared with the female pronucleus. However, this difference disappears before DNA replication begins (Adenot *et al.*, 1997). This may reflect the fact that histones H3 and H4 are acetylated when assembled on to DNA and subsequently become deacetylated (Verreault, 2000).

A second difference is that, although transcriptional activity in the one-cell embryo is quite low compared with subsequent stages, transcription of both endogenous and microinjected genes is higher in the paternal than maternal pronucleus (Aoki *et al.*, 1997; Rastelli *et al.*, 2001). The mechanism underlying this difference is not entirely clear. One clue may come from evidence that plasmid DNA injected into the paternal pronucleus may not become assembled into chromatin. First, expression of the genes injected into the maternal pronucleus, but not the paternal pronucleus, is increased in embryos cultured in the presence of histone deacetylase inhibitors (Wiekowski *et al.*, 1993; Majumder *et al.*, 1997). Second, injection of histones into the paternal pronucleus both reduces injected-gene expression and produces an upregulation in response to the deacetylase inhibitors (Rastelli *et al.*, 2001). However, this explanation seems insufficient, as the paternal DNA appears to be efficiently assembled into chromatin. It is also known that the transcription factors TBP and Sp1 preferentially accumulate in the paternal pronucleus (Worrad *et al.*, 1994). Hence, the higher transcriptional activity in the paternal genome may also reflect the higher abundance of *transacting* factors. The differences described above are not known to persist after the genomes unite in the same nucleus after first mitosis, although factors could associate preferentially with maternally or paternally derived chromatin within the same nucleus.

An unexpected difference between the pronuclei that appears within the first few hours after fertilization and persists during the early embryonic cell cycles is an apparent demethylation of the paternal DNA. In contrast, the maternal DNA remains methylated during this time. Thus, an anti-5-methyl-cytosine antibody (5 mC) stains both male and female chromatin of mouse embryos for the first few hours after fertilization but only the female pronucleus by about 6 h after fertilization (Mayer *et al.*, 2000; Santos *et al.*, 2002). In addition, sequencing of DNA following treatment with sodium bisulphite (which converts unmodified cytosine to uracil but leaves 5-methyl-cytosine unchanged) and PCR amplification indicates that, at the sites analysed, the paternal DNA frequently becomes demethylated whereas the maternal DNA at the same sites does not (Oswald *et al.*, 2000). Demethylation follows histone assembly on the paternal DNA but precedes DNA replication (Santos *et al.*, 2002). These results imply that an ooplasmic activity rapidly demethylates the remodelled paternal DNA after fertilization, but does not affect the maternal DNA residing in the same ooplasm. The molecular nature of this activity is entirely unknown. Moreover, the function of selective paternal demethylation, which has also been observed in embryos of cows, pigs and rats (Dean *et al.*, 2001) but not sheep (Wilmot *et al.*, 2002), remains to be elucidated.

## Chromatin remodelling activity resides in the oocyte

What is the source of the activity that remodels the sperm chromatin at fertilization? Current evidence supports the intuitively attractive idea that it is the oocyte. First, the extent of remodelling of the sperm chromatin depends on the stage of the oocyte at insemination. When oocytes arrested at prophase I are inseminated, the sperm chromatin remains highly condensed, manifesting no morphological changes (although demembrated spermatozoa injected into the oocyte nucleus become dispersed) (Usui and Yanagimachi, 1976; Clarke and Masui, 1986; Maeda *et al.*, 1998). When oocytes undergoing meiotic maturation are inseminated, the sperm chromatin becomes dispersed and then recondenses, often forming individual chromosomes (Clarke and Masui, 1986), and acquires histones (McLay and Clarke, 1997). These changes are similar to those occurring after normal fertilization at metaphase II except that, as maturing oocytes are not activated by sperm penetration, no pronuclei are formed. However, when oocytes are parthenogenetically activated and inseminated several hours later, the sperm chromatin fails to progress beyond an initial dispersion (Usui and Yanigimachi, 1976; Szollosi *et al.*, 1994). Second, when protein synthesis is inhibited during meiotic maturation, sperm chromatin does not progress beyond the stage of initial dispersion (McLay and Clarke, 1997). Third, as assayed by the morphological changes, only a limited number of sperm nuclei can be remodelled within one oocyte (Witkowska, 1981; Clarke and Masui, 1986). Fourth, when prophase I oocytes are enucleated and allowed to mature, they are unable morphologically to remodel sperm chromatin (Balakier and Tarkowski, 1980).

These results indicate that the activity responsible for the early events of remodelling, namely, the initial dispersion and recondensation and the assembly of histones, becomes functional during meiotic maturation. It requires a factor(s) present in the oocyte nucleus and protein synthesis during maturation, and some component of the activity is present in a limited amount. The activity disappears within a few hours after activation of the oocyte. One-cell embryos during the first mitotic division can induce the early events of sperm chromatin remodelling (Maleszewski *et al.*, 1999). Hence, it may be that M-phase cytoplasm is generally permissive for sperm chromatin dispersion and recondensation and assembly of histones. The later events of remodelling, including the extensive decondensation of the chromatin within the male pronucleus and the import of nuclear proteins, however, require transition from M-phase to interphase. This cell-cycle transition is triggered by  $\text{Ca}^{2+}$  oscillations (Ducibella *et al.*, 2002) normally induced in the metaphase II oocyte by a phospholipase C in the fertilizing spermatozoa (Saunders *et al.*, 2002) that initiate destruction of cyclin B (Nixon *et al.*, 2002) and consequent loss of activity of cyclin-dependent kinase 1.

## Oocyte factors implicated in sperm chromatin remodelling

Although remodelling comprises many changes in the structure and composition of the paternal chromatin, the best-understood changes pertain to the replacement of protamines by histones. Protamine removal requires reduction of the intermolecular disulphide bonds and, as the nucleoprotein exchange is sensitive to phosphorylation inhibitors, may also require their phosphorylation (reviewed in Perreault, 1992). The histones are likely supplied by the

oocyte, which accumulates a pool during oogenesis (Wassarman and Mrozak, 1981). Histone assembly into chromatin is an energy-requiring process, and antimycin A, which blocks ATP hydrolysis, prevents paternal chromatin remodelling (Wright and Longo, 1988). In oocytes treated with antimycin A, the paternal chromatin undergoes the initial dispersion but fails to recondense or decondense within a pronucleus. The same result is seen in oocytes penetrated by more than three spermatozoa (Clarke and Masui, 1986). These results taken together reinforce the model that ATP-dependent assembly of oocyte-supplied histones on to the paternal chromatin is a prerequisite for proper remodelling.

In somatic cells, histone assembly onto chromatin requires the activity of chaperone proteins. Results obtained using *Xenopus* indicate that chaperones also mediate histone assembly onto the paternal chromatin at fertilization. *Xenopus* spermatozoa contain histones H3 and H4, but lack histones H2A and H2B, which have been replaced by the sperm-specific proteins, X and Y (Philpott and Leno, 1992). *Xenopus* eggs contain two abundant acidic proteins: nucleoplasmin, which interacts with H2A–H2B dimers and possibly H3–H4 tetramers (Philpott and Leno, 1992; Philpott *et al.*, 2000); and N1, which interacts with H3–H4 tetramers (Dilworth *et al.*, 1987). Nucleoplasmin is essential for histone assembly on to paternal chromatin at fertilization. Purified nucleoplasmin removes proteins X and Y from, and assembles histones H2A and H2B on to, paternal chromatin tetramers *in vitro*. More importantly, immunodepletion of nucleoplasmin from cell-free egg preparations prevents both this nucleoprotein exchange and pronuclear formation. Nucleoplasmin is present in immature oocytes and becomes heavily phosphorylated during meiotic maturation. Phosphorylation substantially increases its histone-assembly activity (Leno *et al.*, 1996). As the paternal chromatin already possesses H3 and H4, N1 is not required for its remodelling at fertilization.

Subsequent studies have identified other proteins from various organisms that can remodel sperm chromatin, as assayed by different criteria (Table 1). However, no proteins with these activities have been described in mammalian oocytes. Such a protein must possess at least two characteristics. First, it must interact directly or indirectly with histones, and potentially with protamines. Second, as remodelling at fertilization occurs on non-replicating DNA, it must be able to assemble histones on to DNA in the absence of DNA replication. One approach to identify mammalian remodelling factors is to search for genes that share sequence similarity with *Xenopus* nucleoplasmin. Two potential homologues have been identified to date: nucleoplasmin 2 (Npm-2) and Npm-3. Npm-2 appears not to play a role in paternal chromatin remodelling at fertilization (M. Matzuk *et al.*, personal communication). Npm-3 protein (MacArthur and Shackelford, 1997) is 42% identical (67% similar) to *Xenopus* nucleoplasmin and its encoding mRNA is expressed in growing and fully grown oocytes (Fig. 2). A second candidate has emerged, somewhat paradoxically, from DNA replication studies. After passage of the replication fork, chromatin assembly occurs in two stages: replication-dependent assembly of the H3–H4 tetramer by chromatin-assembly factor 1, followed by addition of H2A–H2B dimers by nucleosome-assembly protein 1 (NAP-1). First isolated from human extracts and later identified in diverse species, NAP-1 is able to assemble nucleosomes onto unreplicated DNA *in vitro*, as judged by nuclease digestion or supercoiling assays. NAP-1 mRNA is also expressed in oocytes (Fig. 2) and its ability to

assemble nucleosomes in the absence of DNA replication may indicate a function at fertilization.

By microinjecting antisense oligonucleotides, the present authors substantially reduced the concentrations of Npm-3 and NAP-1 mRNAs in mature oocytes. Oocytes were then inseminated to test their ability to assemble histones onto paternal DNA. NAP-1 antisense oligonucleotides had no effect on sperm chromatin remodelling. In contrast, Npm3 antisense oligonucleotides alone, or Npm3 and mNAP antisense oligonucleotides together, prevented histone assembly and formation of the male pronucleus in a significant number of oocytes (Fig. 3; Table 2). These experiments indicate that Npm-3 may serve a function similar to *Xenopus* nucleoplamin during paternal chromatin remodelling at fertilization. In addition, a histone-binding protein that is closely related to N1 (which interacts with H3–H4 tetramers) is encoded by the mammalian *NASP* gene (Richardson *et al.*, 2000). A partial cDNA that matches *NASP* is present in a mouse oocyte cDNA library (<http://lgsun.grc.nia.nih.gov/cDNA>), indicating that it may participate in assembling histones H3 and H4 on to the paternal chromatin.

As described earlier, changes in acetylation of histone also occur during the first cell cycle after fertilization. This indicates that the oocyte contains histone acetyl-transferases (HATs) and histone deacetylases (HDACs). *Xenopus* has been useful in isolating these proteins, because of the large size of the oocytes, and the large maternal stores of histones in comparison with mammalian oocytes. HAT1 has been isolated from frog oocyte extracts in complexes with histone-binding RbAp48 and 14-3-3 proteins (Imhof and Wolffe, 1999). This complex acetylates free histone H4, but not nucleosomal H4, and is localized in the oocyte nucleus, where acetylated free histones are stored. Regarding deacetylases, an oocyte-specific form of HDAC is synthesized during oogenesis and persists until the mid-blastula stage (Ladomery *et al.*, 1997). Maternal HDAC forms complexes with RbAp28–46 and possesses deacetylase activity (Ryan *et al.*, 1999). Similar proteins will likely be found in mammalian oocytes, although pools as large as those found in *Xenopus* may be unnecessary, given that the embryonic genome activates much earlier in mammals than in frogs.

The assembly of histones is only one component of the global remodelling of the sperm chromatin at fertilization. Subsequent chromatin maturation may involve the positioning of nucleosomes as well as the addition of other DNA-binding proteins. For example, ACF, which contains the chromatin-remodelling engine ISWI, was identified from *Drosophila* by its ability, in concert with NAP-1, to assemble and also position nucleosomes (Ito *et al.*, 1999). Members of SWI–SNF are involved in chromatin remodelling and could function at fertilization. Two mammalian homologues, BRM and BRG, are present in mouse eggs and embryos. Deletion of BRM permits viable offspring, possibly due to BRG replacing BRM (Reyes *et al.*, 1998).

## Chromatin remodelling activity develops during oogenesis

As the ability to remodel sperm chromatin resides in the oocyte, it must develop during oogenesis. McLay and Clarke (1997) and McLay *et al.* (2002) used a functional assay to test

the ability of growing and maturing oocytes to assemble histones on to paternal chromatin, which they termed a histone-assembly activity. Fully grown oocytes were inseminated, incubated for different periods, then fixed and stained with DAPI to observe chromatin morphology, and with specific antibodies to monitor histone assembly. With this assay, it was found that when maturing oocytes were inseminated shortly after germinal vesicle breakdown (GVBD), histones were assembled onto the paternal chromatin. However, histone assembly failed to occur when protein synthesis was inhibited during maturation; moreover, this failure could not be rescued by microinjecting histones into the oocyte. These results indicate that histone-assembly activity develops during meiotic maturation through a process requiring new protein synthesis.

This assay was applied to oocytes at different stages of growth (McLay *et al.*, 2002). The results revealed that oocytes acquired histone-assembly activity at the same time that they acquired meiotic competence (defined as the ability to undergo GVBD *in vitro*). However, these activities were separable, as when incompetent oocytes were cultured *in vitro* for several days to allow them to acquire meiotic competence, they did not necessarily also acquire histone-assembly activity. As competent, but not incompetent, oocytes manifest spontaneous  $\text{Ca}^{2+}$  oscillations, incompetent oocytes were treated with  $\text{SrCl}_2$ . This triggered  $\text{Ca}^{2+}$  oscillations in a fraction of the oocytes, and a similar fraction was now able to assemble histones onto paternal chromatin after insemination. This finding indicates that  $\text{Ca}^{2+}$  may promote development of the histone-assembly activity, although the nature of the link is obscure.

These results may be incorporated into the following model (Fig. 4). Growing oocytes accumulate mRNAs encoding factors such as NAP-1 or Npm3 that are required for the development of histone-assembly activity. These mRNAs reach a threshold amount or become translatable at the time of meiotic competence, possibly under the influence of  $\text{Ca}^{2+}$ -regulated events occurring during growth. Upon entry of the oocyte into metaphase of meiotic maturation, the mRNAs are translated to produce functional histone-assembly activity. In this context, it may be noted that the 3'-untranslated region of the mRNA encoding Npm3 contains a putative cytoplasmic polyadenylation element, a sequence in the 3'-untranslated region of other mRNA species that controls their translational activation during maturation (reviewed by de Moor and Richter, 2001). Thus, at the time of fertilization, histone-assembly activity is functional and assembles histones onto the paternal chromatin, completing an early, critical step in the production of a transcriptionally competent paternal genome.

## Paternal chromatin remodelling and somatic nuclear remodelling

Chromatin remodelling by oocytes has attracted particular interest of late because of its potential role in reprogramming somatic nuclei during cloning. Somatic nuclei transferred into an oocyte undergo marked changes in biochemical composition, likely including the import of chromatin remodelling factors (reviewed in Kikyo and Wolffe, 2000). The identification of the molecular players that regulate remodelling of the paternal chromatin at fertilization may lead to better insight into this process. In support of this, nucleoplasmin plays a role in removing linker histones H1 and H1\* from the chromatin of erythrocyte

nuclei in *Xenopus* egg extracts and replacing them with the oocyte-specific variant, H1M, and HMG1 (Dimitrov and Wolffe, 1996). This change produces chromatin similar to that found in early embryos, and also an increase in gene transcription. Perhaps functionally or structurally similar proteins play analogous roles in reprogramming somatic nuclei in mammalian oocytes after nuclear transplantation.

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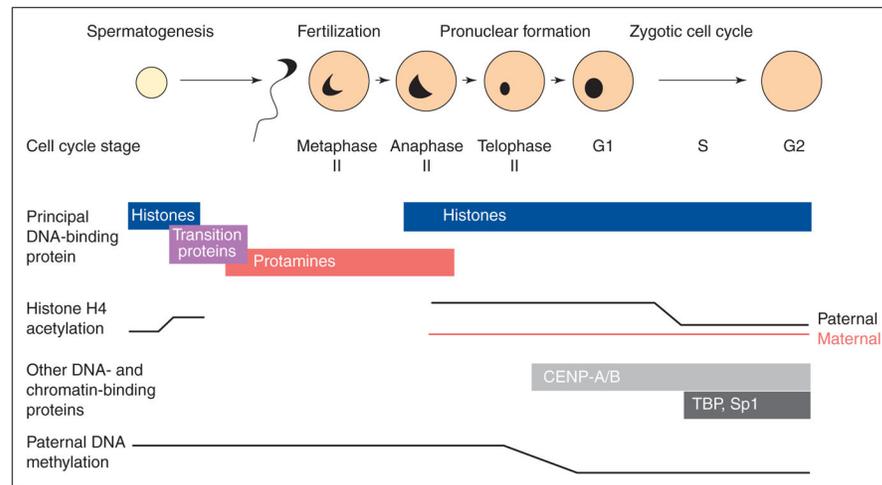
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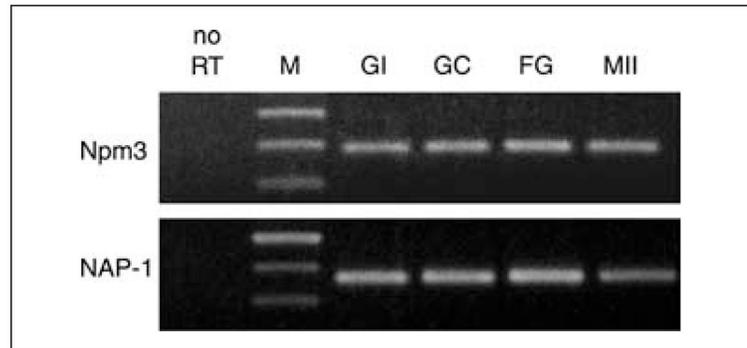
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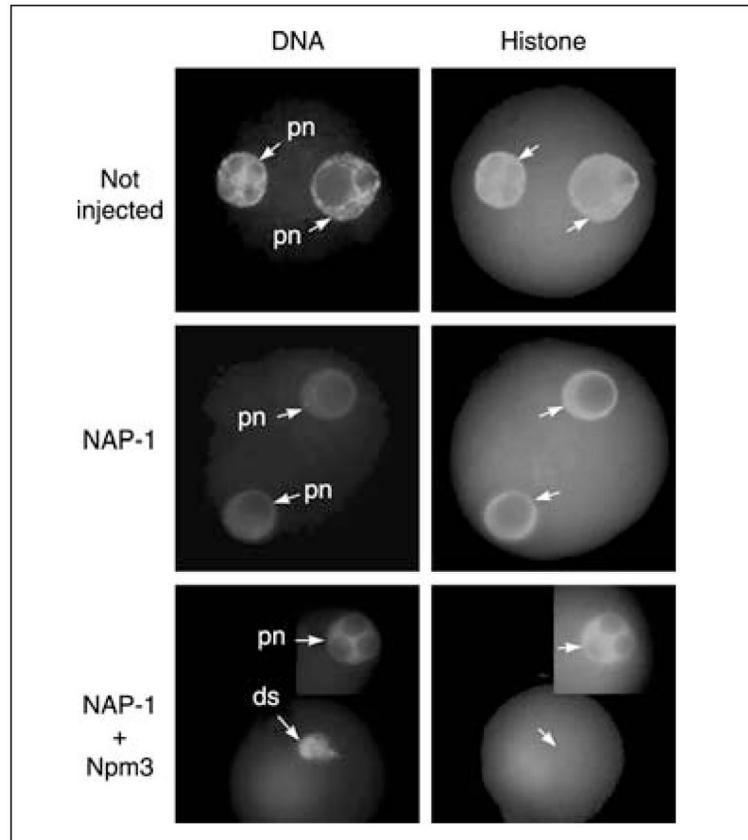
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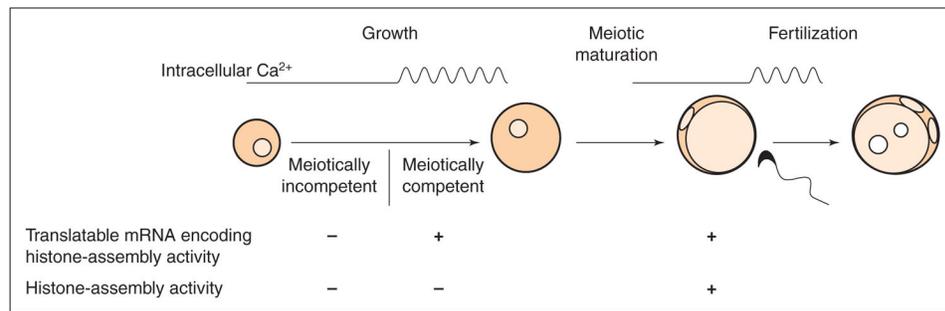
**Fig. 1.** Summary of sperm chromatin remodelling in mammals. During spermatogenesis, sperm histones become acetylated and are replaced by transition proteins, which are then replaced by protamines. After fertilization, the protamines are replaced by oocyte-supplied histones. This replacement occurs as the oocyte completes the second meiotic division and coincides with a transient recondensation of the paternal chromatin. After the protamine–histone exchange, 5-methylcytosine becomes undetectable on the paternal DNA. The paternal and maternal chromatin then decondense extensively within separate pronuclei, and the histones on the paternal chromatin become deacetylated. Other oocyte proteins (centromeric proteins CENP-A/B, and transcription factors TBP and Sp1) imported into the pronuclei further modify the chromatin and DNA replication begins.



**Fig. 2.** Expression of mRNA encoding nucleoplasmin 3 (Npm3) and nucleosome-assembly protein 1 (NAP-1) in mammalian oocytes at different stages of development. RT-PCR was performed using cDNA prepared from 30 oocytes. No RT: reverse transcriptase was omitted from the sample; M: 100-nt molecular size markers; GI: growing, incompetent; GC: growing, competent; FG: fully grown; MII: metaphase II.



**Fig. 3.** Effect of microinjection of antisense oligonucleotides on sperm chromatin morphology and histone assembly after fertilization in mammals. Oocytes arrested at prophase I were injected with oligonucleotides targeting nucleosome-assembly protein 1 (NAP-1) or nucleoplasmin 3 (Npm3), allowed to mature to metaphase II, and then fertilized. The zygotes were fixed and stained 4 h later using 4,6-diamino-2-phenylindole (DAPI) to assess maternal and paternal chromatin morphology (left panels) and an anti-histone antibody to assess histone content (right panels). Top: control uninjected oocyte. Two pronuclei (pn) containing immunodetectable histones are visible. Middle: oocyte injected with NAP-1 antisense oligonucleotides. Two pronuclei containing immunodetectable histone are visible. Bottom: oocyte injected with NAP-1 and Npm3 antisense oligonucleotides. Female pronucleus (inset) is fully developed and contains histones. Paternal chromatin has not progressed beyond the dispersed (ds) state and does not contain detectable histones.

**Fig. 4.**

A model for the development during oogenesis of the activity that assembles histones on to paternal chromatin at fertilization in mammals. During growth, oocytes accumulate mRNAs encoding proteins that mediate DNA replication-independent assembly of histones on to chromatin. These mRNAs become translatable or reach a threshold level at the time that oocytes acquire meiotic competence, and through a process promoted by  $Ca^{2+}$  oscillations. During meiotic maturation the mRNAs are translated to generate histone-assembly activity. Although the activity is functional in maturing oocytes, it normally is not manifested until fertilization activates fully mature metaphase II oocytes.

**Table 1**

## Oocyte factors implicated in sperm chromatin remodelling

Factor	Source	Activity	Reference
Nucleoplasmin	<i>Xenopus</i>	Removal of X and Y from, and assembly of H2A and H2B onto, sperm chromatin	Philpott <i>et al.</i> (2000)
N1	<i>Xenopus</i>	Assembly of H3 and H4 onto new chromatin	Dilworth <i>et al.</i> (1987)
HIRA	<i>Xenopus</i>	Replication-independent nucleosome assembly onto plasmids	Ray-Gallet <i>et al.</i> (2002)
Template-activating factor I $\beta$	<i>Xenopus</i>	Dispersion of sperm chromatin, removal of sperm-specific basic proteins	Matsumoto <i>et al.</i> (1999)
Nucleosome-assembly protein-1	<i>Drosophila</i>	Dispersion of <i>Xenopus</i> sperm chromatin <i>in vitro</i>	Ito <i>et al.</i> (1996)
p22	<i>Drosophila</i>	Dispersion of <i>Xenopus</i> sperm chromatin in fly oocyte extract; release of sperm-specific proteins	Kawasaki <i>et al.</i> (1994)
DF 31	<i>Drosophila</i>	Dispersion of <i>Xenopus</i> sperm chromatin <i>in vitro</i> ; release of sperm-specific proteins	Crevel and Cotterill (1995)
<i>Sésame</i> gene product	<i>Drosophila</i>	Formation of male pronucleus	Loppin <i>et al.</i> (2001)
–	<i>Mytilus</i> (surf clam)	Decondenses spermatozoa in egg extracts	Rice <i>et al.</i> (1995)
–	<i>Spisula solidissima</i> (mussel)	Protein characteristics similar to nucleoplasmin	Herlands and Maul (1994)

**Table 2**

Histone assembly onto paternal chromatin in mammalian oocytes injected with oligonucleotides targeting nucleoplasmin 3 (Npm3) or nucleosome-assembly protein 1 (NAP-1)

Oocyte treatment	Number of experiments	Number of oocytes	Histones on paternal chromatin	
			Detected	Not detected
Not injected	3	23	22	1
Npm3 oligonucleotides	3	29	27	2
NAP-1 oligonucleotides	3	24	16	8
Npm3 + NAP-1 oligonucleotides	3	20	11	9