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A combination of maternal histone variants and chaperones promotes paternal genome activation and boosts somatic cell reprogramming:

A potential role for oocyte-enriched histone variants in nuclear reprogramming and induced pluripotency

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Abstract

The mammalian egg employs a wide spectrum of epigenome modification machinery to reprogram the sperm nucleus shortly after fertilization. This event is required for transcriptional activation of the paternal/zygotic genome and progression through cleavage divisions. Reprogramming of paternal nuclei requires replacement of sperm protamines with canonical and non-canonical histones, covalent modification of histone tails, and chemical modification of DNA (notably oxidative demethylation of methylated cytosines). In this essay we highlight the role maternal histone variants play during developmental reprogramming following fertilization. We discuss how reduced maternal histone variant incorporation in somatic nuclear transfer experiments may explain the reduced viability of resulting embryos and how knowledge of repressive and activating maternal factors may be used to improve somatic cell reprogramming.

Keywords

histone variants; iPS cells; nuclear transfer; reprogramming

Introduction

The mammalian egg and sperm are highly specialized cells with unique epigenetic packaging that must be reprogrammed to create the totipotent cells of the zygote. The non-histone DNA-packaging proteins called protamines constitute up to 90% of human and an even greater percentage of murine sperm chromatin and play an important role in the compaction and transcriptional silencing of paternal nuclei (Box 1) [1, 2]. Protamines are replaced with histones prior to zygotic genome activation and fusion of parental pronuclei. This exchange can be detected within a few minutes of fertilization, and intriguingly, a similar incorporation of maternal histones has also been reported following somatic cell nuclear transfer (SCNT) [3, 4]. With the exception of histone H4, mammalian cells possess

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variants of each canonical histone and each plays diverse roles in DNA repair, chromosome segregation, meiotic recombination, and chromatin packaging [5]. In this essay we highlight the recent findings describing a critical role for maternal histone variants TH2A and TH2B and their chaperone Nucleoplasmin (NPM) in the reprogramming of the paternal genome and somatic cell nuclei, and more broadly discuss how maternal histone variants contribute to reprogramming [6].

H1FOO expression supports genome reactivation after fertilization

The mammalian sperm contains an extremely condensed chromatin structure consisting primarily of protamine proteins (see Box1). Following fertilization, a massive, highly regulated exchange of canonical and variant histones plays a role in resetting chromatin organization of the paternal pronucleus during zygotic genome activation (Fig. 1, Fig. 2A). Previous work highlights the function of a number of histone variants, including H1FOO, H3.3, TH2A and TH2B, during the early stages of development.

In mice, the expression of H1FOO begins at the germinal vesicle stage of oogenesis, and by the MII stage, most linker histones are H1FOO [7, 8]. H1FOO expression ends at the late two-cell or early four-cell stage, coincident with the early wave of zygotic genome activation [7,8]. During fertilization H1FOO's incorporation onto the paternal genome coincides with the removal of sperm protamines as the male genome is chromatinized [9]. A similar result is observed during SCNT (Fig. 2B): H1FOO begins to incorporate onto donor chromatin after several minutes and within one hour, completely replaces somatic histone H1 [7, 8]. Meanwhile, ectopic expression of H1FOO in embryonic stem cells causes a failure of embryoid body differentiation as cells expressing H1FOO continue to express pluripotency-related genes [10]. Nevertheless, at least in certain contexts, H1FOO does not appear to increase efficiency of induced pluripotency [6].

Biochemical analysis indicates H1FOO may impact epigenetic status through its ability to bind hypomethylated DNA and to loosen nearby chromatin [10]. H1FOO generally resembles other H1 family proteins, except for the presence of additional lysine residues. This suggests that additional methylation or acetylation may occur at these sites. Unlike other histones that possess a stem-loop structure at the 3' end of their mRNA transcript, H1FOO is polyadenylated, which indicates differential regulation of H1FOO mRNA stability compared to canonical histones. H1FOO homologs exist in other species such as sea urchin and *Xenopus laevis* [11], while the *Drosophila* H1 variant, dBigH1, is abundantly expressed before cellularization occurs and regulates zygotic gene activation [12]. Taken together it appears that H1FOO-mediated remodeling of chromatin and regulation of zygote genome activation is an ancient and conserved process [13].

Histone H3.3 and its chaperone HIRA are required for reprogramming

Histone H3.3 is another maternal histone variant that regulates activation of the paternal/zygotic genome. Mammalian cells express three different types of histone H3 variants, H3.1, H3.2 and H3.3. The histone variant H3.3 differs from canonical histones H3.1/3.2 by four/five amino acids within the core domain. Unlike its family members, H3.3 can be deposited independently of DNA replication using dedicated chaperones [14]. Soon after fertilization,

the paternal genome rapidly incorporates H3.3, whereas the maternal genome loses most of its H3.3 before it starts to re-accumulate at the late pronuclear stages [15–18]. Such allele bias in H3.3 incorporation timing is suggestive of a pioneering function for histone H3.3 on paternal chromatin for zygote genome activation and the acquisition of totipotency. This idea is supported by studies in both *Xenopus* and *Mus*, where H3.3 incorporation onto donor nuclei is required for successful SCNT [4, 13, 19, 20]. Furthermore, expression of H3.3 containing a single point mutation at H3.3K27, but not H3.1K27, causes developmental arrest by disrupting pericentromeric heterochromatin structure, resulting in derepression of aberrant transcripts from these domains [17], mislocalization of HP1, and ultimately developmental defects [21]. In addition, in the two-cell stage embryo, replication-dependent histones H3.1 and H3.2 exhibit higher mobility associated with totipotency compared to histone H3.3 [22, 23]. Such observations underscore the differences found between histone H3 variants [24].

An important study confirming the maternal requirement of H3.3 in developmental reprogramming however is lacking, namely the genetic deletion of H3.3, which is likely hindered by the fact that H3.3 is encoded by redundant genes, *H3f3a* and *H3f3b*. However, loss of function experiments of H3.3 chaperones further support a function of H3.3 deposition in reprogramming. Notably, progression to the two-cell stage of development is dependent on the histone H3.3 chaperone HIRA in mice; loss of maternal HIRA results in the complete failure of core histone deposition onto the paternal genome and compromised maternal genome reactivation [25]. Developmental arrest appears to be due to the sperm's inability to form a pronucleus and zygotic failure to activate ribosomal RNA transcription [25, 26]. H3.3 loading onto the paternal genome is likely a highly conserved process as *Drosophila* HIRA mutants only display sterility in females and phenotypes for interacting partners are similar to those in mice [25, 27, 28]. The essential role for histone H3.3 in development and SCNT suggests its deletion may also compromise induced pluripotency, an idea further supported by the recent finding of a redistribution of H3.3 during the differentiation of ES cells [29], however, this must be tested empirically. A recent paper extends the role of maternal H3.3, its chaperone HIRA, and nucleosome assembly by demonstrating their requirement for nuclear pore complex formation (NPC) after fertilization [26]. In contrast, another H3.3 chaperone complex, ATRX/DAXX, is responsible for telomere deposition of H3.3, and is dispensable for male pronuclear deposition [30, 31]. These studies highlight the distinct functions of histone H3 variants and their dedicated chaperones during reprogramming, made only more relevant by recent epidemiological studies linking H3.3 and ATRX/DAXX mutations to pediatric brain cancer [32].

Histone TH2A, TH2B, and activated Nucleoplasmin reactivate the paternal genome

Recent findings point to histones TH2A and TH2B (TH2A/B), along with their oocyte-specific chaperone Nucleoplasmin (NPM), as important activators of the paternal genome. Shinagawa et al. describe how oocyte TH2A/B variants incorporate into the paternal pronucleus after fertilization, failing to do so leads to a significant decrease in offspring

viability [6]. This phenotype is contingent on transmission through the maternal germ line; however, embryonic lethality penetrance is incomplete in litters of TH2A/B null dams suggesting the presence of additional compensatory factors for paternal reactivation or a spectrum of sperm responsiveness [6]. Incorporation of maternal TH2B had been previously described, yet compromised viability of offspring was not observed in dams null for TH2B alone [33]. Notably, Montellier et al. demonstrated a compensatory mechanism for TH2B loss in spermatogenesis in which canonical histone H2B is upregulated and chemically modified to produce destabilized nucleosomes [33, 34]. It is thus possible that a similar compensatory mechanism occurs after fertilization of TH2B mutant eggs (and to a lesser extent of TH2A/B mutant eggs) that accounts for their survival. As opposed to paternal reactivation, TH2A/B may only possess a minor, or redundant, role in the activation of the maternal genome. TH2A/B null parthenogenotes show only a modest reduction of viability and slight delay in the display of active chromatin marks by the maternal genome during early pronuclear stages [6].

TH2A/B likely exert their effects by facilitating the transition from repressive to active chromatin marks, wide-spread erasure of DNA methylation, and generally increasing chromatin accessibility. Increased DNase sensitivity upon forced expression of TH2A/B and modeling of the nucleosome structure containing TH2A/B relative to canonical nucleosomes supports this latter notion [6]. Thus TH2A/B seem to share a number of properties with H1FOO and H3.3 in that they are maternally deposited on paternal pronuclei shortly after fertilization, and play an important role in activation of the paternal genome.

In absolute terms, TH2A/B provide only a modest increase in induced pluripotency efficiency (discussed more in the following sections) with the effect dependent on activated NPM [6]. Moreover, NPM alone leads to global decondensation of sperm chromatin, somatic cell nuclei, and an increase success rate in SCNT experiments [35–38]. The synergistic effect observed by Shinagawa and colleagues supports the idea that other maternal factors may aid the process of chromatin remodeling and successful genome reactivation [6].

The ooplasm possesses factors sufficient for reprogramming

Evidence supporting a requirement for maternal histone variants in developmental reprogramming has provided insight into the mechanisms underlying SCNT-based reprogramming and transcription factor-based reprogramming in induced pluripotent stem cells (iPSCs). The ability of mammalian egg cytoplasmic factors to translocate into and reprogram somatic nuclei was first demonstrated in nuclear transfer experiments using enucleated metaphase II-arrested oocytes [39, 40]. In principle, these studies confirmed the findings from amphibians [41] that the egg possesses all of the machinery necessary to reprogram a somatic cell nucleus. However, since SCNT can result in abnormally developed embryos, these studies also show that the donor epigenome contains barriers to complete reprogramming (Box 2).

We speculate that maternal histone variants and their dedicated chaperones are most effective at replacing protamines within compacted sperm nuclei, while their ability to

replace other somatic cell histones is relatively limited. This mismatch between effector and target may account for the different efficiencies of these two processes (Fig. 2B). Indeed, one of the earliest phenotypes associated with SCNT is a failure to fully activate genes that are normally activated at the two-cell (2C) stage of embryo development despite the rapid exchange of histones that has been observed on a macro level by immunofluorescence studies (Fig. 2B) [42]. These 2C genes include a network of genes regulated by the promoters of the murine endogenous retrovirus with leucine tRNA primer (MuERV-L/MERVL) that may play a role in totipotency and the long-term maintenance of pluripotency in ES cell cultures [43–46]. Determining whether the inability to activate 2C genes following SCNT is caused by a genome-wide failure to properly incorporate maternal canonical and variant histones at specific loci will require novel approaches, as existing techniques such as chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) require at minimum tens of thousands of cells.

Maternal histone variants and their incorporation onto the paternal genome upon fertilization may also help explain some of the intriguing and somewhat contradictory results regarding zygotic nuclear proteins and cell cycle compatibility during nuclear transfer. Work from several labs has demonstrated that nuclear transfer requires an oocyte arrested in metaphase of meiosis or fertilized egg in mitosis, as enucleated interphase zygotes were unable to support viability after nuclear transfer [47–50]. This led to the hypothesis that candidate factors involved in reprogramming of somatic nuclei are present within the metaphase cytoplasm of eggs/zygotes and that they then accumulate in the nucleus during interphase. This idea has been further expanded by recent experiments showing that mouse zygotes depleted of the female pronucleus, but not the male pronucleus, can reprogram donor nuclei and can support the full-term development of cloned embryos, suggesting the critical reprogramming proteins are particularly biased to the paternal pronucleus [51]. Interestingly, a recent study has demonstrated that interphase 2C embryos are in fact capable of supporting reprogramming of somatic cells upon enucleation and nuclear transfer as long as donor and recipient cell cycles are carefully synchronized in G0/early G1 [52]. It would be important to test whether this reprogramming by interphase 2C embryos requires zygotic transcription (which initiates prior to the first cleavage), which could perhaps compensate for the loss of maternally derived nuclear proteins upon enucleation. It is also unclear if carefully synchronized donor nuclei could also be successfully reprogrammed by early interphase zygotes after fertilization.

These studies lead us to speculate that the most critical reprogramming factors during SCNT are 1) present in the metaphase-arrested oocyte cytoplasm 2) translocate predominantly to the paternal pronucleus after fertilization and 3) are present in the cytoplasm in 2C embryos (whether maternally or zygotically derived). Thus, oocyte-specific canonical and non-canonical histones and their chaperones are excellent candidates. Histone H1FOO, H3.3, and TH2A/TH2B are abundant in oocytes and replace protamines in the paternal nucleus of the zygote immediately after fertilization; additionally, both core and linker histones have been reported to translocate into the cytoplasm during metaphase [53, 54]. It would be interesting to determine if these histone variants are present in the interphase cytoplasm in early embryos and/or exclusive to the paternal pronucleus. Furthermore, a demonstration of whether the nuclear import of such histone variants into a donor nucleus is dependent upon

nuclear envelope breakdown and/or cell cycle stage compatibility could reconcile the apparent discrepancies of the aforementioned SCNT experiments.

Using developmental reprogramming insights to boost somatic cell reprogramming to iPSCs

The seminal finding by Takahashi and Yamanaka that somatic cells could be reprogrammed into iPSCs by the four OSKM factors (OCT4, SOX2, KLF4, cMYC,) changed our understanding of cellular reprogramming [55, 56]. Defined factors, without additional maternal components, were capable of changing differentiated somatic cells into pluripotent cells capable of forming all structures of the mouse in chimeras and tetraploid embryos [57, 58]. However, our understanding of why transcription factor-mediated reprogramming progresses so slowly has been unclear until recently. The generation of iPSCs occurs rarely and stochastically, and the majority of our assays used to understand the epigenetic basis of reprogramming are population assays, not single-cell assays [59–61]. Numerous advances have begun to unravel the stepwise changes that occur during reprogramming, as well as the major impediments to reprogramming; these findings highlight the role of histone “marks” and the enzymes that catalyze the addition and removal of covalent modifications of histones during the production of iPSCs (reviewed in [62] and [63]). Histone variants themselves are also emerging as key factors regulating this process (Box 2).

Shinagawa et al. have elegantly shown that the oocyte-enriched histone variants TH2A and TH2B are required for efficient reprogramming. The addition of high levels of TH2A/B, and phosphor-mimic NPM can facilitate reprogramming by OSKM or by OK alone (Fig. 2C) [6]. Incorporation of TH2A and TH2B into chromatin leads to a more open chromatin structure, but gene expression studies in reprogramming cells lacking or overexpressing TH2A/B paint a more complicated picture as not all TH2A/B-regulated genes are activated by TH2A/B. Interestingly, TH2A/B are enriched on the X-chromosome relative to autosomes in male reprogramming cells, but it is unclear whether the incorporation onto the X-chromosome or activation of some critical X-linked genes is required for the boost in reprogramming efficiency. Furthermore, whether TH2A/B incorporation into the X-chromosome is required for X-reactivation in female iPSCs is not known, however this may be the case since *Xist* depletion further enhanced the reprogramming boost by TH2A/TH2B and NPM [6]. We believe it would also be interesting to test whether X-biased incorporation of TH2A/B occurs in male and female zygotes, which would be of particular interest since the authors demonstrated a specific requirement for TH2A/B in paternal genome activation (which in male zygotes lack an X-chromosome). Perhaps maternal TH2A/B is less critical in male zygotes than females, which could easily be tested by measuring viability of males and females separately.

Despite the remaining questions regarding the function of TH2A/B histone variants and their chaperone, this study provides proof-of-principle for the use of maternal histone variants to facilitate reprogramming. This study also demonstrates the need for a histone’s corresponding activated histone chaperones such as NPM for efficient incorporation. Recent findings regarding H3.3 and one of its chaperones HIRA and the histone chaperone ASF1 are suggestive and lend further support to this hypothesis [6, 20, 64].

Moving towards an ‘ideal’ reprogramming strategy

Given the chromatin context of somatic and germline cells it will be important to integrate the multiple levels of reprogramming to achieve the tabula rasa so greatly desired by clinicians and basic scientists alike. Transcription factor-based reprogramming has yielded remarkable advances in our understanding of development and disease modeling; however, there are limitations to this method [65–67]. Although efforts into optimal transcription factor dosage and culture conditions have made strides forward towards a perfectly reprogrammed cell, traces of epigenetic memory remain to be addressed [65, 68–73].

The role a subset of variant histones and their associated chaperones play following fertilization are telling of their importance and unique ability to activate restrictive genomes [6, 28]. Perhaps strategies that replace repressive histone variants with other canonical or non-canonical histones would be effective in combination with or even superior to directly modulating enzymes that establish repressive chromatin marks in somatic cells [74–77]. Further research into histone variants, their corresponding chaperones, and their relationship to iPSC derivation will be needed to test these hypotheses. A better understanding of these events and their subsequent incorporation into novel reprogramming schemes will be invaluable as the field advances.

Conclusion

Several histone variants including H3.3, H1FOO, and TH2A/B are enriched in the oocyte and deposited onto the paternal genome by their respective chaperones to replace protamines after fertilization. This exchange of packaging proteins is essential for activation of the paternal genome and subsequent development of healthy embryos. During SCNT, these same histone variants also replace somatic cell histones, although oocyte histone incorporation onto somatic cell nuclei is likely reduced relative to that of protamine-containing sperm nuclei; this may explain the inherent inefficiencies associated with somatic cell cloning. The knowledge of normal developmental reprogramming has led to the discovery that maternal histone variants can facilitate transcription factor-mediated reprogramming of somatic cells to iPSCs, and reminds us that basic developmental biology research leads to important insights that inform the field of regenerative medicine.

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Glossary

iPSCs	induced pluripotent stem cells
SCNT	somatic cell nuclear transfer

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Box 1**Protamines and non-canonical histones package DNA during spermatogenesis**

The ultimate product of spermatogenesis is a highly compacted paternal genome that is delivered to the oocyte upon fertilization. During round spermatid formation and spermatid elongation, chromatin undergoes genome-wide histone hyperacetylation followed by removal of canonical histones[78]. The chromatin state is then reset through the assembly of both core and linker histone variants, transition proteins, and protamines, creating a nearly-quiescent transcriptional state that must be reversed after fertilization [79]. The testis-specific histone variant TH2B facilitates the transition of dissociating core histones into a protamine-packed structure [33]. Depletion of TH2B causes atypical lysine crotonylation and arginine methylation that disrupt nucleosome structure.

The testes specific histone H3 variant H3T is also robustly expressed during human spermatogenesis [80]. Structural analysis indicates H3T-containing nucleosomes are much less stable than canonical H3-nucleosomes, which may indicate that H3T-containing nucleosomes only form intermediate units that are ultimately replaced by protamines[81]. Taken together these studies demonstrate that the presence of histone variants disrupts DNA-histone interactions and leads to a more labile chromatin state—a likely prerequisite for reprogramming.

Box 2**MacroH2A represents a barrier to reprogramming**

Although histones TH2A, TH2B, and their chaperone are examples of factors important for paternal genome activation, not all histone variants share this ability to facilitate reprogramming. MacroH2A is a repressive histone variant associated with differentiation and involved in maintenance of gene silencing and X-chromosome inactivation [82]. After fertilization, maternal macroH2As progressively disappear until just before zygotic genome activation (ZGA) while embryonic macroH2A expression begins only after the 8-cell stage, suggesting a negative role of this histone during reprogramming [83]. Nuclear transfer experiments show macroH2As provide the inactive X-chromosome and pluripotency genes resistance to reprogramming in donor nuclei [84]. Indeed, the removal of macroH2As increases iPSCs formation frequency up to 25-fold [85]. Chromatin immunoprecipitation shows macroH2As occupy pluripotent gene promoters and overlap with the repressive histone mark H3K27me3 [86], which is later replaced by the active histone mark H3K4me3 during reprogramming [87]. Thus, whereas some histone variants are integral to the activation of quiescent genomes, others appear to be just as important in maintaining the repressive somatic cell epigenome thereby limiting reprogramming.

Figure 1.

Histone variant and protamine dynamics through murine fertilization. Maternally contributed histone variants rapidly translocate onto the paternal genome after fertilization and through the pronuclear stages thereafter. The x-axis represents developmental time points and y-axis represents arbitrary units for level of histones, histone variants, and protamines within their respective compartment (see color key and axis labels in figure.) Only the later portions of germline and early portions of zygotic development are depicted for emphasis. Hash marks represent points of interaction between maternal and paternal genetic contribution. GV = germinal vesicle stage, MI = meiosis I, MII = meiosis II, 1C = 1 cell stage, 2C = 2 cell stage.

Figure 2.

The function of maternal histone variants during reprogramming. **A:** The mammalian metaphase II arrested oocyte is enriched with maternal histones variants that are immediately deposited into the paternal pronucleus upon fertilization. These histone variants along with canonical histones replace sperm protamines which package greater than 90% of sperm DNA. Subsequent to this replacement, the zygotic genome is activated, resulting in high levels of zygotic transcripts at the 2-cell (2C) stage. **B:** During somatic cell nuclear transfer, maternal histone variants are deposited rapidly into the somatic cell nucleus, but instead of replacing protamines, they replace somatic histones. As a result, activation of the zygotic/2C genome is compromised, and often results in abnormalities or complete failure of developing embryos. **C:** During standard transcription factor mediated reprogramming with OSKM, cells are slowly and stochastically converted to pluripotent iPS cells, and only in rare iPS cells are zygotic/2C genes expressed (similar to ES cells). This inductive step-wise process can be facilitated by modulating the levels or activity of epigenome modifying enzymes, but also by the addition of maternal factors including the histone variants TH2A/B and its activated chaperone NPM, which may help to make the process more deterministic, like SCNT.