—Mini Review—

Mechanism of Gene Expression Reprogramming during Meiotic Maturation and Pre-Implantation Development

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Introduction

During meiosis and fertilization, gene expression in differentiated gametes is reprogrammed to allow the initiation of a new program from the totipotent zygotic genome. This remarkable transformation entails the deletion of the maternal and paternal gene expression profiles before or just after fertilization. Although reprogramming of gene expression plays an important role in relaying the genome to the next generation, the molecular mechanism of reprogramming remains unknown. Recently, cloned animals were generated in several species by transferring the nuclei of somatic cells into enucleated metaphase II (MII) oocytes [1–8]. The success of these experiments demonstrates that the MII oocyte cytoplasm has the ability to reprogram gene expression, but there is little information on the molecular events in the genome of the transferred nucleus during the reprogramming process. During reprogramming, the gene expression patterns in the differentiated oocytes should be erased, thereby establishing a totipotent gene expression pattern for further development. On the other hand, the discrimination of the paternal and maternal genomes should be maintained during genome reprogramming, since the paternal and maternal genomes are functionally asymmetric in mammals.

In this review, we describe our recent findings on the changes in the epigenetic modifications of differentiated genomes of oocytes during meiosis, and of somatic nuclei after transfer into oocytes, with special emphasis on the mechanism underlying the reprogramming of gene expression. We highlight two aspects of gene expression in the differentiated oocytes. The first involves erasure of information, and the second involves retention of information during meiosis and fertilization, while gene expression is reprogrammed. Some potential applications of these new findings are discussed.

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Erasure of Cell Memory by Histone Deacetylation is Involved in Genome Reprogramming

All eukaryotes contain hundreds of different cell types, each of which has a distinctive set of properties that is defined by a unique pattern of gene expression. In every cell, with the exception of some immune cells, with the same complement of genes, a defined pattern of gene expression is put in place and stabilized by epigenetic mechanisms during cellular differentiation. Since gene expression is inert, and almost all the transcription factors are displaced from the chromosomes during mitosis [9], the stabilized patterns of gene expression persist through the descendent generations by a mechanism that is termed “cell memory” [10–12]. Therefore, it is plausible that the reprogramming of gene expression to generate totipotent zygotes involves the erasure of cell memory, which may be facilitated by changes in epigenetic modifications, such as covalent modifications of DNA-packaging chromatin.

Acetylation of the histone N-tail lysine residues is considered to be a candidate cell memory marker [13]. In chromatin, DNA winds around groups of histone proteins. It has been suggested that combinations of post-translational histone modifications, such as

Table 1. Acetylation of histone lysines during mitosis and meiosis

	GV	GVBD	Egg	1-cell		2-cell		blastocyst		NIH 3T3 cell	
histone/lysine	I*	MI	MII	I	M	I	M	I	M	I	M
H3K9	+	–	–	+	+	+	+	+	+	+	+
H3K14	+	–	–	+	+	+	+	+	+	+	+
H4K5	+	–	–	+	–	+	–	+	–	+	–
H4K8	+	±	±	+	+	+	+	+	+	+	+
H4K12	+	–	–	+	+	+	+	+	+	+	+
H4K16	+	–	–	+	+	+	+	+	+	+	+

Intense and weak fluorescence signals are indicated with + and ±, respectively, and no signal is indicated with –. *I and M indicate interphase and metaphase, respectively.

methylation, acetylation, phosphorylation, and other changes that define the "histone code", constitute an epigenetic marking system that regulates specific gene expression [14–16]. Of those modifications, histone acetylation is the most extensively studied. The acetylation of lysine residues near the N-tails of the histones loosens chromatin packaging, which allows transcription factors to access their enhancer and promoter sequences [17–21], and which correlates with transcriptional activation, whereas histone deacetylation is associated with repression of transcription [22, 23]. Specific lysines on the core histones are acetylated by a series of histone acetylases (HATs), in a process that can be reversed by a diverse series of histone deacetylases (HDACs) [24, 25].

Previously, we suggested that erasure of cell memory maintained by histone acetylation is involved in the reprogramming of gene expression [26]. Immunocytochemistry with antibodies that specifically recognize acetylated lysines 9 and 14 on histone H3 (Ac-H3K9 and Ac-H3K14) and acetylated lysines 5, 8, 12 and 16 on histone H4 (Ac-H4K5, Ac-H4K8, Ac-H4K12 and Ac-H4K16, respectively) has shown that acetylation of these lysines is decreased to negligible levels during meiotic maturation in mouse oocytes, whereas most of these lysines remain to be acetylated during mitosis in pre-implantation embryos and NIH3T3 cells (Table 1). Deacetylation was also observed in the somatic nuclei that were transplanted into the MII stage oocytes (Fig. 1). These observations support the idea that the acetylation of histones H3 and H4 is involved in the inheritance of gene expression patterns, and that global deacetylation promotes the reprogramming of gene expression. Although it has been established that the cytoplasm of the MII stage oocyte is able to reprogram the gene expression patterns of transplanted somatic nuclei [7, 27], little is known about the molecular processes that the transplanted nuclei undergo in the oocytes during the process of reprogramming. Our results suggest that the oocyte cytoplasm resets a program for gene expression in the transplanted nuclei by deacetylating histones.

Histone acetylation has also been reported to play important roles in various chromatin-based processes, such as DNA replication and chromosome segregation. Increased acetylation accelerates the timing of DNA replication [28, 29]. Hyperacetylated histones that are induced by aberrant control of HDAC activity contribute to chromosomal instability with impaired mitotic progression [30] and defective chromosome condensation, which results in poor sister chromatid separation [31]. Furthermore, global histone deacetylation during meiosis appears to be essential for proper chromosome alignment on the meiotic spindle, since chromosomal hyperacetylation causes abnormal chromosome alignment at MII [32]. The relationship between these phenomena and genome remodeling, which is regulated by histone acetylation, remains to be elucidated.

Recently, it has been shown in somatic nuclear cloning that certain properties of the donor cells are retained in the cloned embryos [33]. A two-fold increase in the rate of development to blastocysts was achieved in cloned embryos by the use of nuclei from myoblast donor cells that were cultured in the medium generally used for the donor cells, as compared to donor cells that were cultured in the medium used for embryo culture. In these embryos, the myoblast specific gene *GLUT-4* was still expressed during early development. This example of remnant gene expression from the donor cell indicates that cell memory is not erased sufficiently during somatic nuclear cloning.

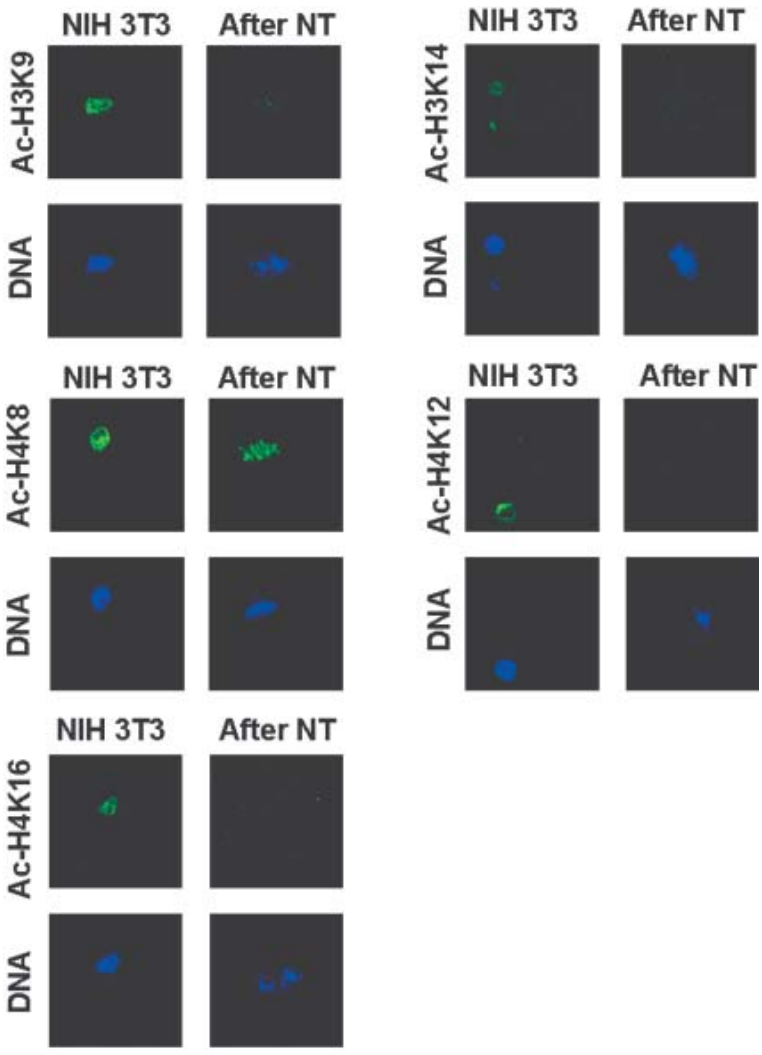

Fig. 1. Deacetylation of histones in somatic nuclei transplanted into MII stage oocytes. The enucleated mouse MII oocytes were transplanted with interphase nuclei of NIH3T3 cells. The NIH3T3 cells were embedded in the perivitelline space of the enucleated oocytes (NIH3T3). Two hours after electrofusion (After NT), the oocytes were subjected to immunostaining with the antibodies to each of the acetylated lysine residues of histone H3 and histone H4. The samples were counterstained with DAPI to visualize the DNA.

Histone H3 Lysine 9 Methylation is an Epigenetic Marker for Parental Genome Origin

Parental genomes are not functionally equivalent in mammals, and contribution from both the maternal and paternal genomes is required for normal development [34]. The differences in the properties of paternal and maternal genomes may be attributable to differences in the epigenetic modifications to their genomes. It is presumed that the information that discriminates the parental origin of the genomes in the gametes is maintained during reprogramming, whereas the information on differentiated gene expression pattern, i.e., cell memory, is erased. This asymmetry in parental genomes is embodied by genomic imprinting and X chromosome inactivation. Genomic imprinting is an epigenetic mechanism, by which the expression of certain genes, i.e., imprinted genes, is dependent on whether the allele is inherited from the mother or the father [35]. DNA methylation is essential for silencing

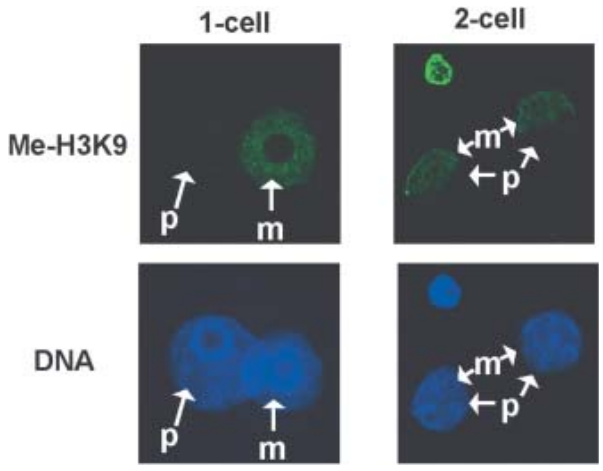

Fig. 2. Asymmetric histone H3 methylation of lysine 9 in pre-implantation mouse embryos. Mouse 1-cell and 2-cell embryos were subjected to immunocytochemistry with the antibody to methylated histone H3 Lys 9 (Me-H3K9). The antibody was localized with FITC-conjugated secondary antibodies, and the DNA was stained with DAPI (DNA). The arrows indicate the paternal (p) and maternal (m) genomes. In the mouse 2-cell embryos, the paternal and maternal genomes are topologically compartmentalized in the same nucleus.

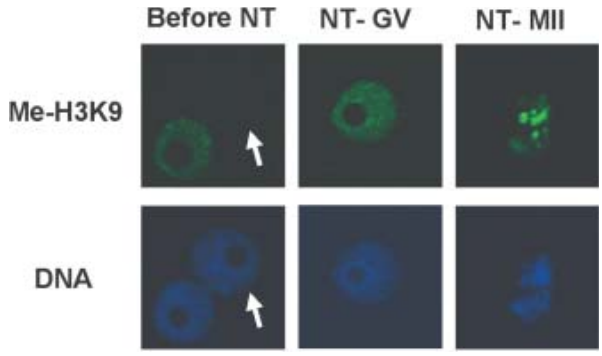

Fig. 3. Histone H3 lysine 9 methylation in male pronuclei that were transplanted into enucleated GV stage or MII stage oocytes. The reconstructed oocytes were produced by transferring male pronuclei (indicated by arrowheads; Before NT) into enucleated oocytes at the GV stage (NT-GV) or MII stage (NT-MII). The antibody that recognizes the methylated lysine 9 (Me-H3K9) on histone H3 was localized with FITC-conjugated secondary antibodies, and the DNA was stained with DAPI (DNA).

the imprinted genes, and at most imprinted loci there are key regulatory sequences that are methylated on one of the parental alleles only [36]. In female mammals, one of the two X chromosomes is converted from the active euchromatic state to the transcriptionally inert heterochromatin, in a process known as X chromosome inactivation [37, 38]. Preferential inactivation of the paternal X chromosome occurs in extra-embryonic tissues, such as the placenta [39]. A growing body of evidence suggests that there are direct and indirect links between gene silencing and epigenetic modification of the genome [22, 40, 41].

Histone H3 lysine 9 (H3K9) methylation has been linked to gene silencing and the assembly of heterochromatin [42–48]. Methylation of H3K9 is carried out by the enzymes SUV39H and G9a [49, 50], and this provides a binding site for heterochromatin-associated proteins, such as HP1, through the chromodomain in mammals [14, 51]. In addition, a higher level of H3K9 methylation is observed in the inactive X chromosome than in the active X chromosome [52, 53]. It has been suggested that H3K9 methylation correlates with DNA cytosine methylation, and that DNA cytosine methylation acts downstream of H3K9 methylation [44, 54, 55].

Recently, we have reported that asymmetric H3K9 methylation between paternal and maternal genomes is an epigenetic marker for discriminating the parental origins of genomes, and that post-fertilization changes in H3K9 methylase activity are involved in the maintenance of the information about genome origin [56]. As shown in Fig. 2, the asymmetric methylation of lysine 9 on histone H3 (Me-H3K9) between paternal and maternal genomes occurs in early pre-implantation embryos. A very weak or no methylation signal was detected in the paternal genome, whereas distinct methylation signals were detected in the maternal genomes. This asymmetry existed through the 1-cell to the 2-cell stage and disappeared at the 4-cell stage, at which time *de novo* H3K9 methylation occurs in both the paternal and maternal genomes [56]. The undermethylated state of H3K9 in the paternal genome is maintained by an active process that depends on gene expression and protein synthesis, since *de novo* methylation in the paternal genome occurs when either transcription or protein synthesis is inhibited [56]. In addition, *de novo* H3K9 methylation of male pronuclei is observed when they are introduced into enucleated MII oocytes (Fig. 3), which suggests that H3K9 methylase is active in MII oocytes but not in embryos after fertilization. Thus, H3K9 is methylated only in the maternal genome, which is present in the MII oocytes before fertilization, and is not methylated in the paternal genome, which is absent. These results suggest that

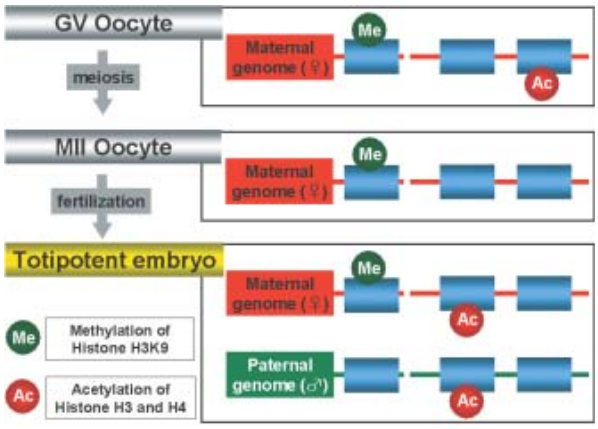

Fig. 4. Erasing cell memory and maintaining information on the parental origin of genomes during genome reprogramming. Histone acetylation is displaced from the maternal meiotic genome. After fertilization, asymmetric H3K9 methylation generated by "on-off" regulation of H3K9 methylase activity functions as an epigenetic marker for discriminating the paternal and maternal genomes in totipotent embryos.

H3K9 is an epigenetic marker of parental genome origin during early pre-implantation development.

The finding that the levels of H3K9 methylation increased in paternal pronuclei that were transferred into MII stage oocytes (Fig. 3) is consistent with the previous observation that the expression of some imprinted genes is disrupted in cloned animals [57–59]. Since asymmetric H3K9 methylation may be disrupted due to an increase in the H3K9 methylation of transferred nuclei, it is conceivable that mechanisms for discriminating parental genome origin during early embryogenesis could malfunction, to some extent, in cloned embryos. As a consequence, disruption of parent-specific mono-allelic expression of imprinted genes would occur in the cloned animals. Imprinted genes are implicated in the function of the placenta, and placental abnormalities represent the most common abnormal phenotypes in cloned animals. Therefore, the disruption of asymmetric H3K9 methylation in cloned embryos may be the intrinsic and inherent reason why the efficiency of somatic cloning remains extremely low.

Conclusion and Outlook

We propose molecular mechanisms for the reprogramming processes that erase the information on gene expression patterning in differentiated oocytes, i.e., cell memory, but which maintain the information on the parental origins of the genome (Fig. 4). The cytoplasmic environment of the MII oocyte erases cell memory markers, such as histone acetylation, thereby transforming the differentiated genome into a totipotent zygotic genome. On the other hand, the information about the parental origin of the genome is maintained *via* altered H3K9 methylase activity in the oocyte cytoplasm during fertilization.

Understanding the reprogramming mechanisms will facilitate the efficient production of cloned animals. Although success in somatic nuclear cloning has been reported in many species and by several research teams, the rate of success in obtaining viable offspring remains extremely low (<5%) to date [60]. In addition, live cloned animals show a variety of morphological and metabolic abnormalities, such as respiratory failure, placental dysfunction, and large offspring syndrome [61, 62]. How can the inefficiency of somatic cloning be overcome? Several strategies to improve the efficiency of somatic cloning have been attempted, such as changing the culture medium and conditions [63], seeking optimal strains for cloning [64], modifying enucleation timing [65] and pretreating the donor cells with mitotic cell extract [66], but none of these trials have led to significant increases in somatic cloning efficiency. Manipulating the cytoplasmic factors that are involved in histone deacetylation and H3K9 methylation of the recipient oocytes may provide novel approaches for addressing this issue. For instance, the production of transgenic animals that have more HDAC genes linked to the ZP3 promoter, which is activated specifically during oocyte growth [67], may be a way to improve the efficacy of somatic nuclear cloning. The increased expression of HDAC in the recipient oocytes would contribute to more efficient erasure of the gene expression pattern memory in the donor cells. Transient inhibition of H3K9 methylase during meiotic maturation is an alternative method for improving cloning efficiency. As mentioned above, discrimination between genomes of paternal and maternal origin may be disrupted in cloned embryos due to *de novo* H3K9 methylation of transferred somatic nuclei in the recipient MII oocytes. Therefore, the inhibition of H3K9 methylase activity *via* RNA interference (RNAi) in MII oocytes may solve this problem. RNAi is a conserved cellular mechanism that promotes sequence-specific mRNA degradation triggered by double-stranded RNA (dsRNA), and it has proven to be a powerful tool for inhibiting gene expression in several experimental model systems, including *Arabidopsis*, *C. elegans* and *Drosophila* [68, 69]. The RNAi pathway starts when the

RNase III enzyme (Dicer) cuts dsRNA into small interfering RNAs (siRNAs), which subsequently target homologous mRNAs for destruction, resulting in gene silencing [70]. This sequence-specific interference mediated by dsRNA has been described recently for the mouse oocyte and pre-implantation embryo [71–74]. These studies and our observations raise the possibility that the efficiency of somatic cloning can be improved through the specific inhibition of H3K9 methylation with RNAi in the enucleated oocyte.

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