Changes in histone acetylation during mouse oocyte meiosis

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We examined global changes in the acetylation of histones in mouse oocytes during meiosis. Immunocytochemistry with specific antibodies against various acetylated lysine residues on histones H3 and H4 showed that acetylation of all the lysines decreased to undetectable or negligible levels in the oocytes during meiosis, whereas most of these lysines were acetylated during mitosis in preimplantation embryos and somatic cells. When the somatic cell nuclei were transferred into enucleated oocytes, the acetylation of lysines decreased markedly. This type of deacetylation was inhibited by trichostatin A, a specific inhibitor of histone deacetylase (HDAC), thereby indicating that HDAC is able to deacetylate histones during meiosis but not during mitosis. Meiosis-specific deacetylation may be a consequence of the accessibility of HDAC1 to the chromosome, because HDAC1 colocalized with the chromosome during meiosis but not during mitosis. As histone acetylation is thought to play a role in propagating the gene expression pattern to the descendent generation during mitosis, and the gene expression pattern of differentiated oocytes is reprogrammed during meiosis to allow the initiation of a new program by totipotent zygotes of the next generation, our results suggest that the oocyte cytoplasm initializes a program of gene expression by deacetylating histones.

Introduction

The acetylation of nuclear core histones is thought to play important roles in various cellular functions. The biological importance of histone acetylation was first suggested after the discovery that the amount of acetylation is well correlated with the level of transcription (Allfrey et al., 1964). Subsequently, numerous works have shown the important roles of histone acetylation in the regulation of gene expression. Histones H3 and H4 are hyperacetylated in active genes, whereas they are underacetylated in silent genes (Clarke et al., 1993; O’Neill and Turner, 1995; Grunstein, 1997). The acetylation of histone H4 occurs initially at lysine 16 (K16), and then at K8 or K12, and ultimately at K5 (Turner and Fellows, 1989; Thorne et al., 1990; O’Neill and Turner, 1995). Thus, the acetylation of K5 reflects the hyperacetylated state in histone H4 and is strongly correlated with the active states of the genes (Grunstein, 1997; Mizzen and Allis, 1998; Turner, 1998). Chromatin immunoprecipitation experiments have shown that histone H4 is hyperacetylated in the promoter regions of active genes (Kuo et al., 1998; Chen et al., 1999; Parekh and Maniatis, 1999; Shestakova et al., 2001). Experiments of this type have also shown that lysines 9 and 14 on histone H3 (H3/K9 and H3/K14, respectively) are acetylated in the promoter region when the gene is activated (Agalioti et al., 2002). Recently, it has been demonstrated that distinct histone modification patterns function as recognition codes for the recruitment of different transcription factors upon transcriptional activation (Agalioti et al., 2002). The acetylation of H4/K8 is involved in the recruitment of the SWI/SNF complexes to chromatin, whereas the acetylation of H3/K9 and H3/K14 is required for the recruitment of transcription factor IID. Thus, the histone code may function as an epigenetic marker that is directly associated with transcriptional activation.

Recently, it has been suggested that histone acetylation is also involved in cellular functions other than gene expression (Kurdistani and Grunstein, 2003). A line of evidence has revealed that heterochromatin formation involves histone acetylation. Sir2 deacetylates H4/K16 to form heterochromatin in the chromosomal region near the telomeres (Imai et al., 2000; Kimura et al., 2002; Suka et al., 2002). The acetylation of histones is also involved in the regulation of DNA replication. DNA within the chromosomal domain...
The acetylation of lysine 14 on histone H3 at interphase (I) and metaphase (M). Oocytes, preimplantation embryos, and NIH 3T3 cells were immunostained with the anti-acetyl histone H3/lysine 14 (H3/K14) antibody. GV, oocytes at the GV stage; GVBD 3 h, oocytes after a 3-h incubation without IBMX in the first meiosis; Egg, oocytes at MII; 1-cell (I), one-cell embryos at interphase; 1-cell (M), one-cell embryos at the M phase; 2-cell (I), two-cell embryos at interphase; 2-cell (M), two-cell embryos at the M phase; Blastocyst, blastocyst-stage embryos; NIH 3T3 (I), NIH 3T3 cells at interphase; NIH 3T3 (M), NIH 3T3 cells at the M phase. Arrows indicate the condensed mitotic chromosomes in the blastocysts. Each sample was counterstained with DAPI to visualize the DNA. The acetylation of lysine 9 on histone H3 is shown in Fig. S4, available at http://www.jcb.org/cgi/content/full/jcb.200303047/DC1.

Figure 1. Acetylation of lysine 14 on histone H3 at interphase (I) and metaphase (M). Oocytes, preimplantation embryos, and NIH 3T3 cells were immunostained with the anti-acetyl histone H3/lysine 14 (H3/K14) antibody. GV, oocytes at the GV stage; GVBD 3 h, oocytes after a 3-h incubation without IBMX in the first meiosis; Egg, oocytes at MII; 1-cell (I), one-cell embryos at interphase; 1-cell (M), one-cell embryos at the M phase; 2-cell (I), two-cell embryos at interphase; 2-cell (M), two-cell embryos at the M phase; Blastocyst, blastocyst-stage embryos; NIH 3T3 (I), NIH 3T3 cells at interphase; NIH 3T3 (M), NIH 3T3 cells at the M phase. Arrows indicate the condensed mitotic chromosomes in the blastocysts. Each sample was counterstained with DAPI to visualize the DNA. The acetylation of lysine 9 on histone H3 is shown in Fig. S4, available at http://www.jcb.org/cgi/content/full/jcb.200303047/DC1.

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them may involve changes in histone acetylation. However, there is no knowledge of these changes during meiosis.

In this study, to investigate the involvement of histone acetylation in the regulation and function of meiosis, we examined the changes in the acetylation patterns of various lysine residues in oocyte histones during meiosis and compared these acetylation patterns with those seen during mitosis in postfertilization embryos. In addition, we examined the localization of HDAC in the oocytes and embryos, because HDAC1 is expressed in Xenopus oocytes (Ryan et al., 1999) and may play a role in the deacetylation of histones during meiosis.

**Results**

**Histone acetylation at the M phase during meiotic maturation and preimplantation development**

The acetylation levels of various lysine residues on histones H3 and H4 were examined in NIH 3T3 cells, mouse oocytes, and preimplantation embryos. Immunocytochemistry with specific antibodies against acetylated lysines 9 and 14 on histone H3 (Ac-H3/K9 and Ac-H3/K14) and acetylated lysines 5, 8, 12, and 16 on histone H4 (Ac-H4/K5, Ac-H4/K8, Ac-H4/K12, and Ac-H4/K16) showed intense fluorescence signals in the nuclei of the NIH 3T3 cells at interphase (Figs. 1 and 2 for H3/K14 and H4/K12, respectively; see Figs. S1–S4 for the others, available at http://www.jcb.org/cgi/content/full/jcb.200303047/DC1). All of these signals, except for that of H4/K5, were also observed in metaphase chromosomes, as described previously, although decreases were seen for both the Ac-H3/K9 and Ac-H4/K5 signals in the previous study (Kruhlak et al., 2001). These results reinforce the idea that the acetylation of these lysines marks the transcriptionally active chromatin domains for postmitotic reactivation (Kruhlak et al., 2001). Intense fluorescence signals for all of the antibodies were detected in the germinal vesicles (GVs) of the full-grown oocytes that were arrested at the G2 phase in the first meiotic cell cycle. However, all of these signals disappeared in the condensed chromosomes of the oocytes 3 h after transfer to 3-isobutyl-methylxanthine (IBMX)–free medium, except in the case of H4/K8, for which the signal intensity persisted, albeit at a low level. In our system, the oocytes underwent germinal vesicle breakdown (GVBD) and entered the M phase in the first meiosis within 1 h of transfer to IBMX-free medium (Sobajima et al., 1993). The fluorescence signals for all of the acetylated lysines, with the exception of Ac-H4/K8, were also absent from the oocytes at metaphase II (MII) of the second meiosis. A weak signal for Ac-H4/K8 persisted in these oocytes. However, all of the signals reappeared after

![Figure 2](http://www.jcb.org/cgi/content/full/jcb.200303047/DC1)
fertilization and were detected continuously both at interphase and M phase in the one-cell, two-cell, and blastocyst embryos, except for the Ac-H4/K5 signal, which disappeared in the metaphase chromosomes. It seems unlikely that the loss of signal in the meiotic chromosome was an artifact caused by changes in structure or masking by meiosis-specific proteins, as the chromosomes of the MII-stage oocytes stained intensely with the antibody against methylated lysine 9 on histone H3 (Arney et al., 2002; unpublished data). Thus, all of the lysine residues were deacetylated only during meiosis, except for H4/K5, which was also deacetylated during mitosis. The acetylation states of the various lysine residues in the oocytes, embryos, and NIH 3T3 cells are summarized in Table I.

### Table I. Acetylation of histone lysines during mitosis and meiosis

<table>
<thead>
<tr>
<th>Histone/lysine</th>
<th>GV</th>
<th>GVBD</th>
<th>Egg</th>
<th>1-Cell</th>
<th>2-Cell</th>
<th>Blastocyst</th>
<th>NIH 3T3 cell</th>
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Intense and weak fluorescence signals are denoted with + and ±, respectively, and no signal with −.

*I* and *M* indicate interphase and metaphase, respectively.

Histone acetylase and deacetylase activities during meiosis

Although previous studies showed that HDACs were unable to deacetylate histones in the condensed chromatin of somatic cells at the M phase (Kruhlak et al., 2001), the absence of oocyte acetylation suggests that the oocyte cytoplasm deacetylates histones during meiosis. To verify this hypothesis, we conducted in situ histone acetylase (HAT) and HDAC assays using trichostatin A (TSA), which is a specific inhibitor of HDACs (Yoshida et al., 1990; Kruhlak et al., 2001).

First, to examine HAT activity, the MII-stage oocytes were incubated with TSA, and changes in the acetylation levels of H3/K14 and H4/K12 were examined. The oocytes were incubated for 14 h after isolation from the ovaries, at which time the oocytes reached the MII stage and showed no signals for the anti–Ac-H4/K12 or anti–Ac-H3/K14 antibodies (Fig. 3 for H4/K12; see Fig. S5 for H3/K14, available at http://www.jcb.org/cgi/content/full/jcb.200303047/DC1), as shown in Figs. 1 and 2. The oocytes were matured in vitro for 14 h in TSA (E), and either washed to remove the TSA followed by culturing for 3 h (G), or cultured continuously in TSA for 3 h (F). Each sample was counterstained with DAPI to visualize the DNA. The results of analysis for histone H3/lysine 14 are shown in Fig. S5, available at http://www.jcb.org/cgi/content/full/jcb.200303047/DC1.
K12 or anti–Ac-H3/K14 antibodies (Fig. 3 for H4/K12; Fig. S5 for H3/K14). These results indicate that HAT is unable to acetylate the histones in MII-stage oocytes. Similarly, we examined HAT activity in oocytes during the first meiosis by incubating the oocytes with TSA for 4–7 h after transfer to IBMX-free medium. The results showed that HAT did not function in the first meiosis (Fig. 4 for H4/K12; see Fig. S6 for H3/K14, available at http://www.jcb.org/cgi/content/full/jcb.200303047/DC1).

Second, to examine HDAC activity, the GV-stage oocytes were incubated with TSA and allowed to resume meiosis by transfer to IBMX-free medium. TSA did not affect the percentage of oocytes that underwent GVBD or reached MII at 2 or 14 h, respectively, after transfer to IBMX-free medium. Intense signals were detected for the anti–Ac-H4/K12 and anti–Ac-H3/K14 antibodies after incubation with TSA for 14 h (Fig. 3 for H4/K12; Fig. S5 for H3/K14). The oocytes were washed free of TSA and reincubated without TSA for 3 h, by which time the signals for the anti–Ac-H4/K12 and anti–Ac-H3/K14 antibodies had disappeared completely (Fig. 3 for H4/K12; Fig. S5 for H3/K14). In contrast, these signals were retained in oocytes that were maintained in medium with TSA for the additional 3-h incubation. These results show that HDAC deacetylates H4/K12 and H3/K14 in MII-stage oocytes. A similar experiment was conducted to examine HDAC activity in oocytes during the first meiosis. In this instance, the oocytes were incubated with TSA for 4 h, and then without TSA for 3 h after transfer to IBMX-free medium. The results show that HDAC also functions during the first meiosis (Fig. 4 for H4/K12; Fig. S6 for H3/K14).

Deacetylation of histones in nuclei transferred into MII oocytes

It has been established that the cytoplasm of the MII-stage oocyte is able to reprogram the gene expression pattern of transferred somatic nuclei (Wilmut et al., 1997; Rideout et al., 2001). If we assume that the acetylation of histones H3 and/or H4 is involved in the inheritance of gene expression patterns, then these histones from somatic nuclei should be deacetylated in oocytes. To address this issue, the nuclei of NIH 3T3 cells were transferred into enucleated MII-stage oocytes, and changes in the histone acetylation levels were examined. 2 h after the transfer, the interphase nucleus underwent premature chromosome condensation. In this chromosome, the signals for the antihistone antibodies decreased dramatically (Fig. 5 A for H3/K14 and H4/K12; see Fig. S7 for the others, available at http://www.jcb.org/cgi/content/full/jcb.200303047/DC1). When the intensity of the fluorescence signal was quantified, the signal for Ac-H4/K16 decreased to an undetectable level, those for Ac-H3/K9, Ac-H3/K14, and Ac-H4/K12 decreased dramatically to a negligible level, and that for Ac-H4/K8 significantly decreased (P \leq 0.01) but persisted at an appreciable level (see Fig. S8, available at http://www.jcb.org/cgi/content/full/jcb.200303047/DC1). However, intense signals were detected for all of the antibodies in the embedded nucleus, which lies between the plasma membrane and zona pellucida of the oocyte, and where the intact nucleus is not exposed to the cytoplasm. The decreases in the acetylation levels in the oocytes were probably due to deacetylation by HDAC, as TSA treatment inhibited the deacetylation of the transferred nuclei. When the enucleated oocytes were cultured with TSA, the transferred nuclei underwent premature chromosome condensation in the same manner as the control oocytes that were cultured without the inhibitor. Intense signals for both the anti–Ac-H3/K14 and anti–Ac-H4/K12 antibodies were observed (Fig. 5 A) in this type of chromosome. Thus, MII-stage oocytes remove the acetyl groups from the chromatin-associated histones of transferred nuclei. Histone deacetylation also occurred in the condensed chromosomes that were transferred from the NIH 3T3 cells. The chromosomes were isolated from NIH 3T3 cells, which were arrested at the M phase by nocodazole, and transferred...
into MII-stage oocytes. Although the signals for Ac-H3/14 and Ac-H4/K12 were reduced to low levels 2 h after transfer, intense signals remained in the oocytes that were treated with TSA (Fig. 5 B). The intense signals were also retained in the embedded chromosome between the plasma membrane and zona pellucida. Thus, the cytoplasm of the MII-stage oocytes could remove the acetyl groups from H3/K14 and H4/K12 in the transferred chromosomes. These results provide direct evidence that HDAC deacetylates the condensed chromosomes in oocytes.

Localization of HDAC1 during meiosis and mitosis
It has been shown in *Xenopus laevis* that HDACm, which is a homologue of mammalian HDAC1 (Taunton et al., 1996; Bartl et al., 1997; Ryan et al., 1999), is synthesized during oogenesis and accumulates in the GVs of growing oocytes. HDACm was localized around the internal margins of the nuclei and was not associated with the chromosomes (Ryan et al., 1999).

Therefore, we examined the localization of HDAC1 in oocytes during meiosis to investigate the potential involvement of HDAC1 in histone deacetylation. The NIH 3T3 cells were immunostained with the anti-HDAC1 antibody and costained with DAPI. HDAC1 was detected in the interphase nuclei, although no HDAC1 was detected in the mitotic chromosomes (Fig. 6). In the GV-stage oocytes, HDAC1 was detected in GVs, but in the area where the DNA was not localized. However, after 3 h of incubation without IBMX, HDAC1 colocalized with the chromosomes.

Discussion
In this work, we demonstrated that the acetylation levels of various lysine residues on histones H3 and H4 were markedly decreased, both in oocytes during meiosis and in somatic nuclei that were transferred into enucleated oocytes. HDAC altered these lysines during meiosis but not during mitosis. This meiosis-specific deacetylation may be due to HDAC1 accessibility to the chromosome, as it colocalizes with the chromosome only during meiosis and not during mitosis.

The acetylation of core histones is thought to play roles in the propagation of genomic function information from one cell generation to the next by retaining this information during the mitotic phase (Turner, 2000; Jenuwein and Allis, 2001), as well as in genome activation by producing conformational changes in the chromatin structure, which render the DNA accessible to transcription factors during interphase (Turner, 1998), and by providing binding surfaces for recruiting transcriptional activators (Kurdistani and Grunstein, 2003). In this study, we examined the acetylation...
states of various lysine residues on histones H3 and H4 and found that all of the lysine residues examined, except for H4/K5, remained in the acetylated state during mitosis in preimplantation embryos and NIH 3T3 cells (Figs. 1 and 2; Table I). These results suggest that acetylation of the lysine residues (other than H4/K5) is involved in the propagation of genomic function through the preservation of stable acetylation during mitosis. Because H4/K5 was completely deacetylated during mitosis, both in the preimplantation embryos and somatic cells, the acetylation of this residue does not appear to be involved in this type of mechanism. It has been proposed that the components of the system propagating genomic function information simply transmit information and do not have a direct effect on chromatin structure (Turner, 1998). This is consistent with our results, as it has been suggested that the acetylation of H4/K5 affects chromatin structure. This residue is the last of the four lysines in the NH₂-terminal tail of histone H4 to be acetylated, thereby creating the hyperacetylated state of histone H4, in which the chromatin structure is sufficiently relaxed to allow transcription factor access (Turner, 1998).

Although gene expression patterns are inherited by the next generation in differentiated somatic cells, the gene expression patterns of immature oocytes, which are also differentiated cells, need to be reprogrammed, and the new program is established before or after fertilization. This reprogramming of gene expression probably involves global epigenetic modifications. Our results show that the global acetylation level of the histones decreases dramatically during meiosis (Figs. 1 and 2), which suggests that histone deacetylation is involved in the reprogramming of gene expression. This hypothesis is supported by the results of the nuclear transfer experiments. Recent success in the production of cloned animals has shown that the cytoplasm of MII-stage oocytes is able to reprogram the gene expression patterns of the transferred somatic nuclei (Wilmut et al., 1997; Wakayama et al., 1998; Rideout et al., 2001). In our study, the histone acetylation levels decreased markedly in the somatic nuclei that were transferred into the enucleated oocytes (Fig. 5).

When differentiated cells proliferate, the information on the active genes is transmitted faithfully to the daughter cells, thereby maintaining the lineage-specific program of gene expression in the descendant generations. As most of the transcription factors are displaced from the chromosomes and the genes are silent during mitosis (Hershkovitz and Riggs, 1995; Martinez-Balbas et al., 1995), it has been suggested that a mechanism exists to maintain the lineage-specific program of gene expression. This mechanism is called “cell memory” (Weintraub, 1985; Riggs and Pfeifer, 1992), and it suggests that certain epigenetic modifications remain as markers of cell memory in the chromosomes during mitosis (Jeppesen, 1997; Turner, 1998). Histone acetylation has been proposed as a candidate epigenetic marker (Jeppesen, 1997; Grunstein, 1998; Turner, 2000, 2002). Studies using an antibody against acetylated H4/K12 have shown that the active X chromosome is acetylated to a higher degree than the inactive X chromosome in the inter-
In chromosome segregation during mitosis in fission yeast (Ekwall et al., 1997). The meiosis-specific underacetylation of histones H3 and H4 observed in this study may be involved in meiosis-specific chromosomal segregation.

We have shown that HDAC deacetylates histones, but only during meiosis. Although HDAC retains its activity in cultured cells during mitosis, it lacks access to the mitotic chromosomes (Kruhlak et al., 2001). In *Xenopus laevis*, HDACm, which is a homologue of the mammalian HDAC1, is localized in the GV but is not associated with the chromosomes in the GV-stage oocytes. HDAC is active during meiosis, but its localization has not been examined in meiotic oocytes (Ryan et al., 1999). We have shown that HDAC1 does not colocalize with the DNA in GV-stage murine oocytes (Fig. 6). However, during meiosis, HDAC1 colocalized with the meiotic chromosomes in the oocytes, whereas it was displaced from the mitotic chromosomes in the NIH 3T3 cells (Fig. 6). These results suggest that HDAC1 has access to the chromosomes only during meiosis, which suggests a possible mechanism for meiosis-specific deacetylation of histones. However, it was reported previously that yeast RPD3, which is a homologue of the mammalian HDAC1, deacetylates all of the lysine residues, except for H4/K16 (Roth et al., 2001; Suka et al., 2001; Narlikar et al., 2002). Therefore, it seems likely that another HDAC is involved in the deacetylation of H4K16 in mouse oocytes. Further study of the expression and function of various HDACs should provide important clues for uncovering the mechanism by which histone deacetylation is involved in the regulation and function of meiosis.

Materials and Methods

Preparation of oocytes and embryos

Fully-grown oocytes were collected from 3-wk-old ddY or B6CDF1 mice (SLC) and incubated in Whitten’s medium with 0.2 mM IBMX, as described previously (Sobajima et al., 1993). The oocytes were subsequently cultured in Whitten’s medium (Whitten, 1971) without IBMX to allow meiotic maturation.

The embryos were prepared by in vitro fertilization using MI-stage oocytes from 1-wk-old female ddY or B6CDF1 mice and sperm from mature male ICR mice (SLC) and cultured as described previously (Fuchimoto et al., 2001). Embryos at the interphase of the one-cell stage, M phase of the one-cell stage, interphase of the two-cell stage, and M phase of the two-cell stage and blastocysts were collected 10, 15, 30, 36, and 96 h after insemination, respectively.

Nuclear transfer

The enucleation of oocytes was conducted as described previously (Kim et al., 2002). The nuclei of NIH 3T3 cells were introduced into the enucleated oocytes by electrofusion, using a DC pulse of 1,500 V/cm for 20 μs in 300 mM mannitol that contained 0.1 mM MgSO4, 0.1 mg/ml polyvinyl alcohol, and 3 mg/ml bovine serum albumin. During the transfer of condensed chromosomes, the NIH 3T3 cells were synchronized at metaphase by treatment with nocodazole (0.5 μg/ml; Sigma-Aldrich) for 16 h and labeled with Hoechst 33342 (Sigma-Aldrich). After UV irradiation, only those cells that contained condensed chromosomes were selected as nuclear donors.

Immunostaining and quantification of fluorescence intensity

The oocytes, embryos, and NIH 3T3 cells were immunostained with antibodies against acetylated lysines 5, 8, 12 (Upstate Biotechnology), and 14 (Upstate Biotechnology) of histone H3, and HDAC1 (Santa Cruz Biotechnology, Inc.). The cells were fixed with 3.7% paraformaldehyde for 30 min and then permeabilized with 0.5% Triton X-100 for 10 min, followed by incubation with the anti–acetyl histones for...
References


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