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Changes in histone acetylation during mouse oocyte meiosis

Jin-Moon Kim, Honglin Liu, Mayuko Tazaki, Masao Nagata, and Fugaku Aoki

Department of Integrated Biosciences, Graduate School of Frontier Sciences, University of Tokyo, Chiba 277-8562, Japan

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 chromatins, 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

J.-M. Kim and H. Liu contributed equally to this work.

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Address correspondence to Fugaku Aoki, Department of Integrated Biosciences, Graduate School of Frontier Sciences, University of Tokyo, Chiba 277-8562, Japan. Tel.: 81-4-7136-5424. Fax: 81-4-7136-3698. E-mail: aokif@k.u-tokyo.ac.jp

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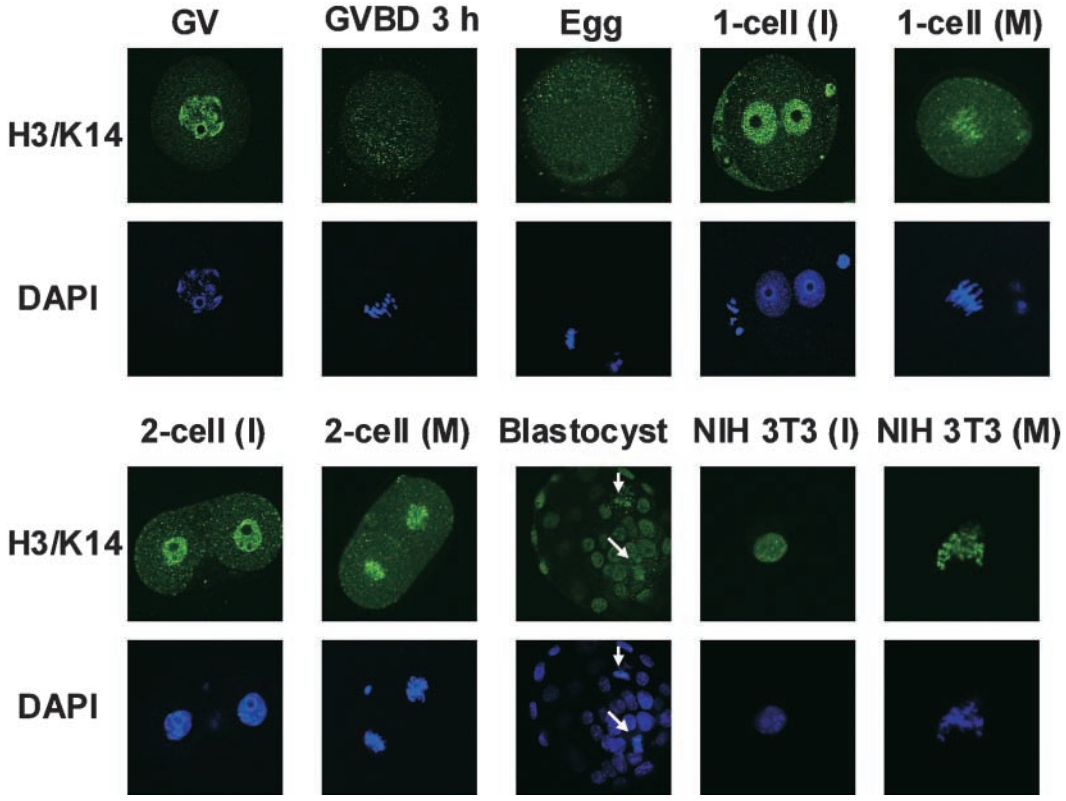

Figure 1. **Acetylation of lysine 14 on histone H3 at interphase (I) and metaphase (M).** Oocytes, preimplantation embryos, and NIH 3T3 cells were immunostained with the anti–acetyl histone H3/lysine 14 (H3/K14) antibody. GV, oocytes at the GV stage; GVBD 3 h, oocytes after a 3-h incubation without IBMX 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.

of late replication is associated with deacetylated histone H4 (Jeppesen and Turner, 1993; Zhang et al., 2002). Increased acetylation accelerates the timing of DNA replication (Aoki and Schultz, 1999; Vogelauer et al., 2002). Consequently, histone acetylation has been revealed to play important roles in various chromatin-based processes.

In addition to these functions during interphase, it is thought that histone acetylation plays a role during mitosis. It may function as an epigenetic marker to propagate the information on genomic function from one generation of cells to the next (Strahl and Allis, 2000; Turner, 2000; Jenuwein and Allis, 2001). Because gene expression is inert and almost all of the transcription factors are displaced from the chromosomes during mitosis (Martinez-Balbas et al., 1995), the inheritance of gene expression patterns by the next generation should require the transfer of some epigenetic markers for active genes. Histone acetylation has been proposed as a potential inherited epigenetic marker, as the acetylation of histones H3 and H4 persists during the mitotic phase (Kruhlak et al., 2001), and higher levels of acetylation are maintained in active than in inactive X chromosomes during mitosis (Jeppesen and Turner, 1993; Migeon et al., 1994). In addition, histone acetylation seems to be involved in the mechanism regulating chromosome segregation. Increased acetylation by histone deacetylase (HDAC)* inhibitor induces chromosome loss after mitosis in fission yeast (Ekwall et al., 1997).

Although it has been suggested that histone acetylation plays important roles during mitosis, little is known about its involvement in the mechanism or function of meiosis. There are many events that specifically occur during meiosis, but not mitosis, e.g., successive M phase without intervening DNA replication, pairing of homologous chromosomes, asymmetric cell division, etc. In addition to these meiosis-specific events, the function of meiosis in the regulation of gene expression differs from that in mitosis. The oocyte genome must be reprogrammed during meiosis, which is followed by fertilization, to allow the remarkable transformation from differentiated oocytes into the totipotent embryos of the next generation (Schultz et al., 1999). Recent success in the production of cloned animals by somatic nucleus transfer has also shown that the oocyte cytoplasm has the ability to reprogram gene expression (Wilmut et al., 1997; Wakayama et al., 1998; Rideout et al., 2001). Most of these meiosis-specific events and functions probably involve meiosis-specific changes in chromatin structure, and some of

*Abbreviations used in this paper: GV, germinal vesicle; GVBD, germinal vesicle breakdown; HAT, histone acetylase; HDAC, histone deacetylase; IBMX, 3-isobutyl-methylxanthine; MII, metaphase II; TSA, trichostatin A.

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

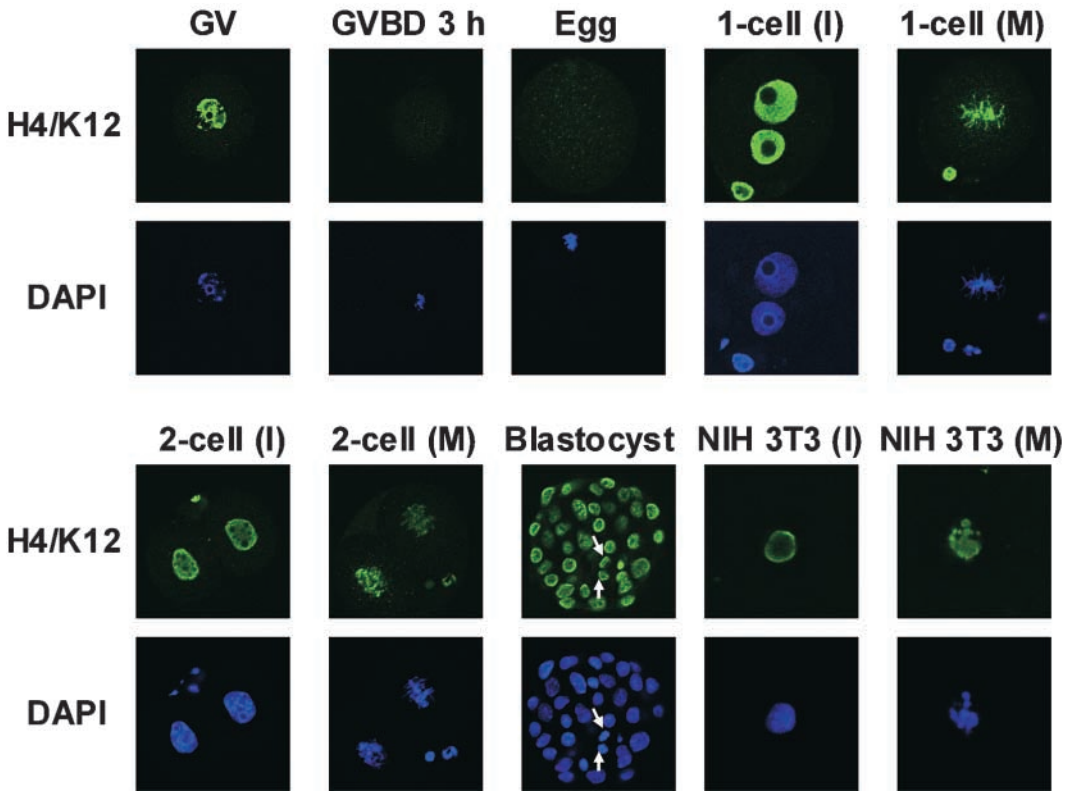

Figure 2. **Acetylation of lysine 12 on histone H4 at interphase (I) and metaphase (M).** Oocytes, preimplantation embryos, and NIH 3T3 cells were immunostained with the anti–acetyl histone H4/lysine 12 (H4/K12) antibody. GV, oocytes at the GV stage; GVBD 3 h, oocytes after a 3-h incubation without IBMX 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. The arrows indicate condensed mitotic chromosomes in the blastocysts. Each sample was counterstained with DAPI to visualize the DNA. The acetylation of lysines 5, 8, and 16 on histone H4 is shown in Figs. S1–S3, respectively, available at http://www.jcb.org/cgi/content/full/jcb.200303047/DC1.

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

Histone/lysine	GV	GVBD	Egg	1-Cell		2-Cell		Blastocyst		NIH 3T3 cell	
	I[a]	MI	MII	I	M	I	M	I	M	I	M
H3/K9	+	−	−	+	+	+	+	+	+	+	+
H3/K14	+	−	−	+	+	+	+	+	+	+	+
H4/K5	+	−	−	+	−	+	−	+	−	+	−
H4/K8	+	±	±	+	+	+	+	+	+	+	+
H4/K12	+	−	−	+	+	+	+	+	+	+	+
H4/K16	+	−	−	+	+	+	+	+	+	+	+

Intense and weak fluorescence signals are denoted with + and ±, respectively, and no signal with −.
[a]I and M indicate interphase and metaphase, respectively.

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.

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 then transferred to medium that contained TSA and incubated for a further 3 h. The histone acetylation levels should have been increased by HAT acetylation of the histones, while HDAC activity should have been inhibited by TSA. However, no signals were obtained for either the anti–Ac-H4/

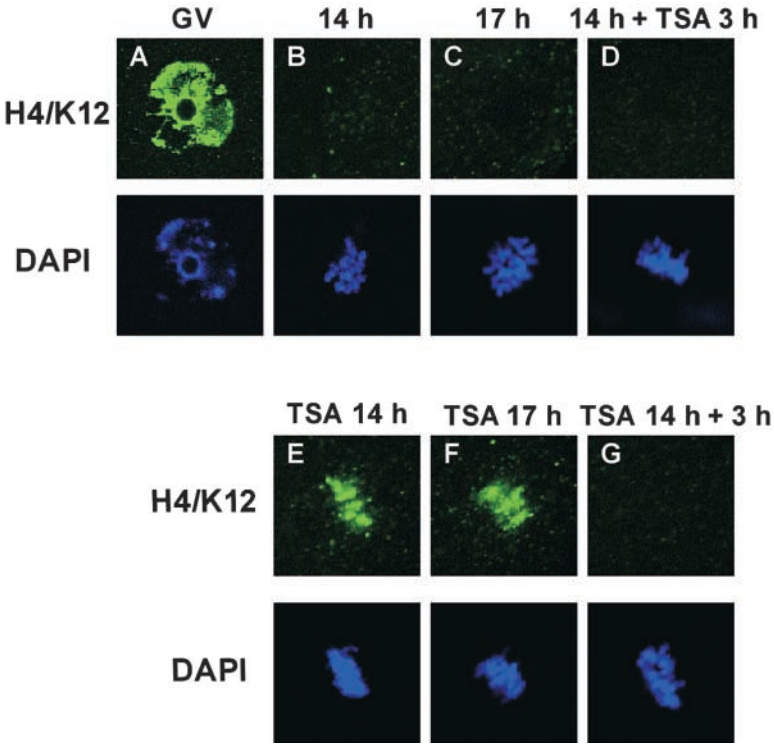

Figure 3. **In situ analysis of the histone acetyltransferase and deacetylase in MII-stage oocytes.** Oocytes at the GV and MII stages were immunostained with the anti–acetyl histone H4/lysine 12 (H4/K12) antibody. The GV-stage oocytes (A) were cultured in vitro for 14 h to mature into the MII stage (B) and were subsequently cultured for 3 h with TSA (D) or without TSA (C). 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.

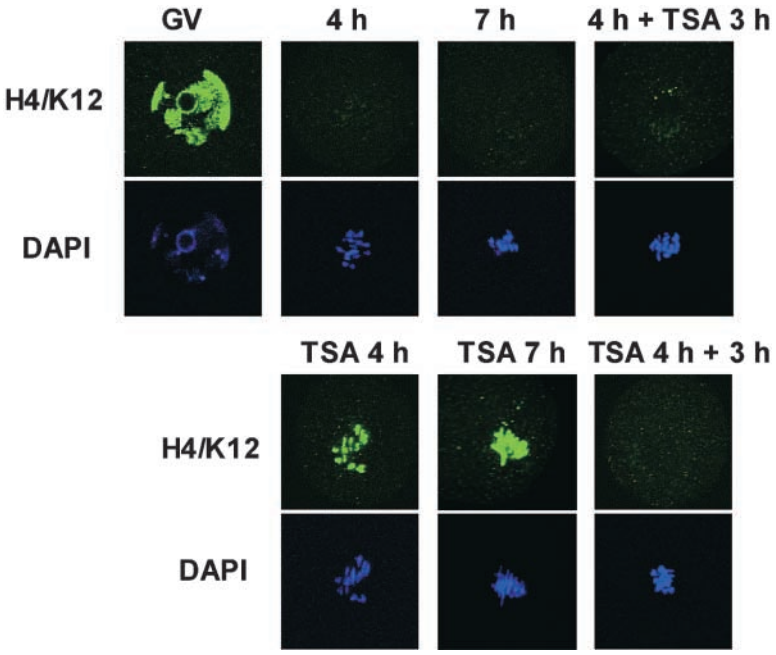

Figure 4. **In situ analysis of the histone acetyltransferase and deacetylase in oocytes during the first meiosis.** Oocytes in the first meiosis were immunostained with the anti–acetyl histone H4/lysine 12 (H4/K12) antibody. The GV-stage oocytes (A) were cultured in vitro for 4 h to induce the first meiosis (B) and subsequently cultured for 3 h with TSA (D) or without TSA (C). Oocytes that were undergoing the first meiosis after in vitro culture for 4 h in TSA (E) were either washed free of TSA and cultured for a further 3 h (G), or cultured continuously in TSA for 3 h (F). Each sample was counterstained with DAPI. The results of analysis for histone H3/lysine 14 are shown in Fig. S6, 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 ($P < 0.01$) 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

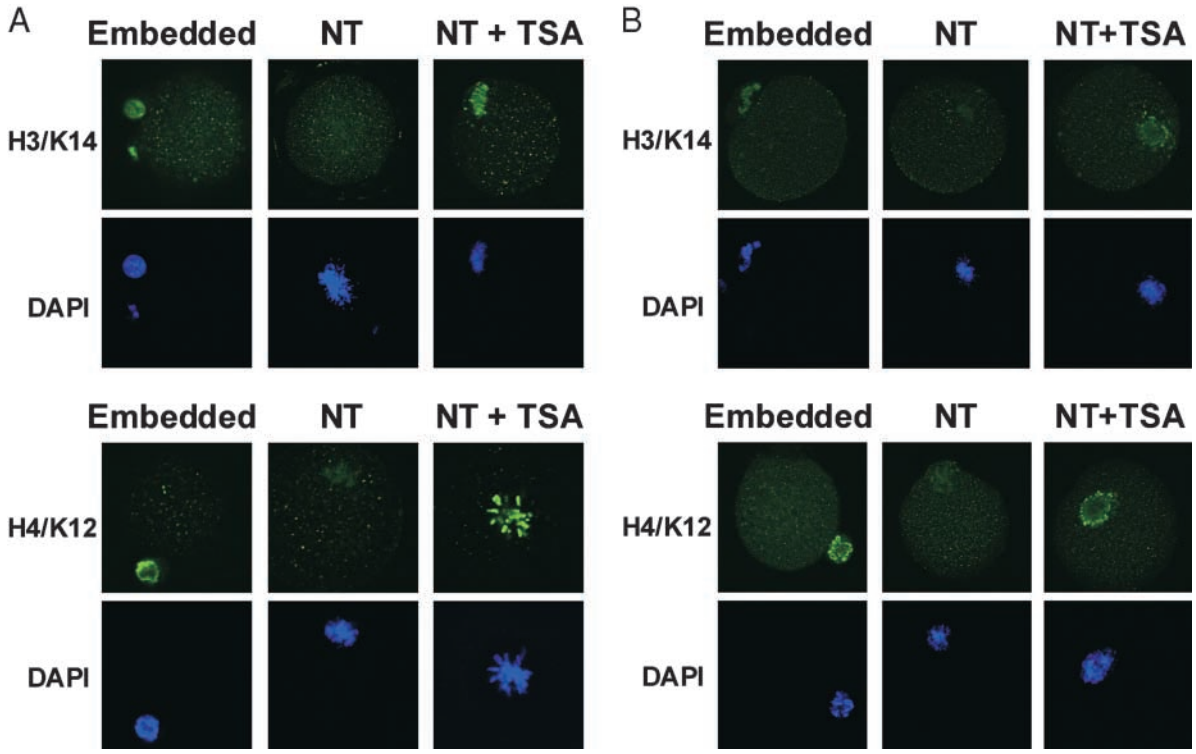

Figure 5. **Deacetylation of histones in transferred nuclei and chromosomes.** The enucleated oocytes were transplanted with interphase nuclei (A) or metaphase chromosomes (B) of NIH 3T3 cells. NIH 3T3 cells were embedded in the perivitelline space of enucleated oocytes (embedded). Both during and after the electrofusion procedures, the enucleated oocytes and reconstructed embryos were treated with either 75 nM TSA (NT+TSA) or without TSA (NT). 2 h after electrofusion, the oocytes were subjected to immunostaining with the anti–acetyl histone H3/lysine 14 (H3/K14) or the anti–acetyl histone H4/lysine 12 (H4/K12) antibodies. Each sample was counterstained with DAPI. The deacetylation of lysine 9 on histone H3 and lysines 8 and 16 on histone H4 in the transplanted nucleus is shown in Fig. S7, available at http://www.jcb.org/cgi/content/full/jcb.200303047/DC1. The fluorescence signal of the antibody was quantified, and the results are shown in Fig. S8, available at http://www.jcb.org/cgi/content/full/jcb.200303047/DC1.

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

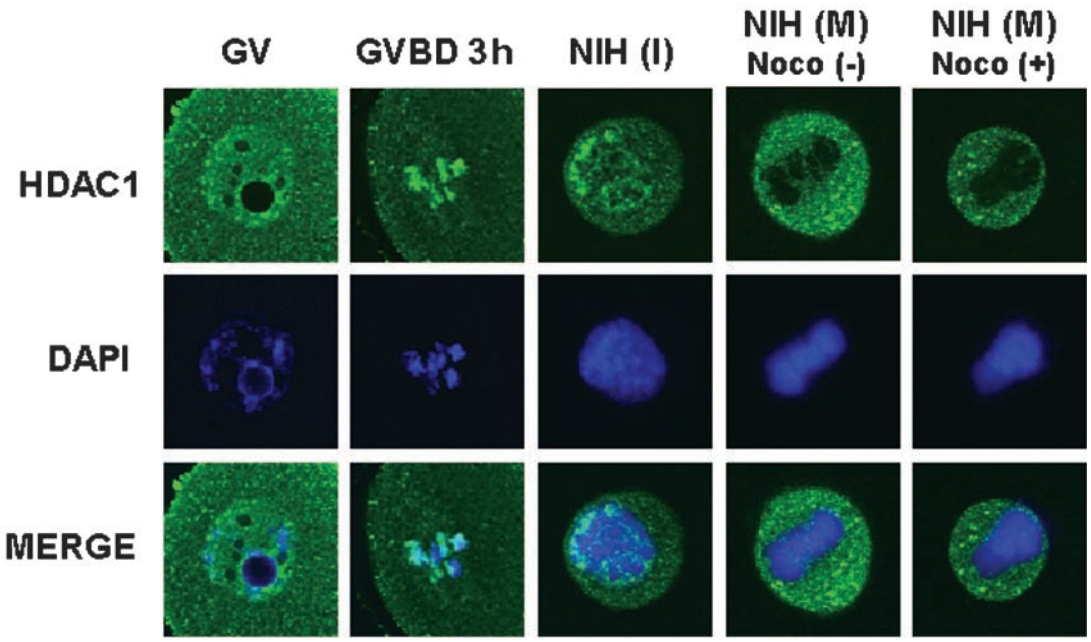

Figure 6. **Localization of HDAC1 during meiosis and mitosis.** The GV-stage oocytes (GV), oocytes that were incubated for 3 h in IBMX-free medium (GVBD 3 h), and NIH 3T3 cells at interphase (NIH [I]), natural mitotic phase (NIH [M] noco [−]), or nocodazole-arrested mitotic phase (NIH [M] noco [+]) were immunostained with the antibody against HDAC1 (top) and costained with DAPI (middle). The bottom row shows the merged images from the top and middle rows.

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 NH_2-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 a previous report showed that the global acetylation level of H3/K9 was reduced during the mitotic phase (Kruhlak et al., 2001), the H3/K9 acetylation level was not reduced in our experiment. Although the previous report described a reduction in the acetylation level, the extent of that reduction was not discussed. Therefore, it is possible that the level of reduction is low, and below the threshold of detection in our experimental system.

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 descendent 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-

phase nuclei, and that this difference is maintained during mitosis (Jeppesen and Turner, 1993; Migeon et al., 1994). Whereas lysine 5, which is the last of the four lysines in the NH_2-terminal tail of histone H4 to be acetylated, and whose acetylation has been shown to be closely linked to active gene expression, is deacetylated during the M phase, H4/K8, H4/K12, H4/K16, and H3/K14 remain acetylated globally in the chromosome in human fibroblasts (Kruhlak et al., 2001). Furthermore, in a genetic screen of budding yeast mutants, in which the telomere-proximal genes that are heritably active in the wild type were locked into the silent state, the mutants showed prominent reductions in their H4/12 acetylation levels (Smith et al., 2002). These studies suggest that the acetylation of these lysines represents a marker of cell memory. This idea is supported by our results, which showed that all of the lysine residues were deacetylated in the oocytes during meiosis and in the somatic nuclei that were transferred into enucleated oocytes, in which genome reprogramming occurred. In the process of genome reprogramming, acetylated lysines should be deacetylated to erase any information on active genes, i.e., cell memory. Therefore, genome reprogramming may involve the erasing of cell memory by histone deacetylation to create the undifferentiated or totipotent zygotes of the next generation.

The Ac-H4/K8 signal remained in oocytes during meiosis and in the nucleus of NIH 3T3 cells that were transferred into oocytes, although the intensity of the signal was decreased significantly. This level of decrease should be sufficient for reprogramming the genome. However, it is possible that H4/K8 remains acetylated and is involved in the remodeling mechanism. It has been suggested that acetylation of this residue mediates the recruitment of the SWI/SNF complex, which is a chromatin-remodeling ATPase (Peterson and Workman, 2000; Agalioti et al., 2002). It has also been reported that the chromatin-remodeling ATPase ISWI plays an important role in the remodeling of the somatic nucleus in *Xenopus* egg extracts (Kikyo et al., 2000). Therefore, H4/K8 acetylation may play a role in chromatin remodeling by recruiting the chromatin-remodeling ATPase during meiosis or after fertilization.

There are also many meiosis-specific events other than the reprogramming of gene expression in oocytes, e.g., successive M phase without intervening DNA replication, pairing of homologous chromosomes, and asymmetric cell division. Most of these events probably involve meiosis-specific changes in chromatin structure, and some may involve changes in histone acetylation. For instance, it has been proposed that histone acetylation is associated with DNA replication. DNA within the chromosomal domain of late replication is specifically associated with deacetylated histone H4 (Jeppesen and Turner, 1993; Zhang et al., 2002). An increase in the acetylation of histones near the late replication origin accelerates the timing of DNA replication (Vogelauer et al., 2002). Therefore, DNA replication appears to be repressed in the chromosomes with deacetylated histones. Therefore, deacetylation after GVBD may be a necessary prerequisite for the absence of DNA replication during meiosis. It has also been suggested that histone acetylation is associated with centromere function. Treatment with TSA induced a hyperacetylated state in centromeric chromatin and caused defects 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 MII-stage oocytes from 3-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 $MgSO_4$, 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 16 (Santa Cruz Biotechnology, Inc.) of histone H4, lysines 9 (Cell Signaling Technology) 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

1 h at room temperature or overnight at 4°C. The antibodies that bound to the oocytes were probed with FITC-conjugated anti–mouse or anti–rabbit IgG (Jackson ImmunoResearch Laboratories). Counterstaining was conducted with DAPI to visualize the DNA. Fluorescence was detected using the Leica spectral confocal scanning system.

The intensity of fluorescence was quantified as previously described (Aoki et al., 1997). In brief, the pixel value of fluorescence was measured within a constant area from five different regions of chromosomes and five different regions of cytoplasm, and the average cytoplasmic value was subtracted from the average chromosomal value.

Online supplemental material

The supplemental figures (Figs. S1–S8) are available at http://www.jcb.org/cgi/content/full/jcb.200303047/DC1. All the chemicals used were purchased from Wako Chemical Inc., unless otherwise specified, and were of analytical grade.

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References

Allfrey, V.G., R. Faulkner, and A.E. Mirsky. 1964. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc. Natl. Acad. Sci. USA.* 51:786–794.

Aoki, F., and R.M. Schultz. 1999. DNA replication in the 1-cell mouse embryo: stimulatory effect of histone acetylation. *Zygote.* 7:165–172.

Aoki, F., D.M. Worrad, and R.M. Schultz. 1997. Regulation of transcriptional activity during the first and second cell cycles in the preimplantation mouse embryo. *Dev. Biol.* 181:296–307.

Agalioti, T., G. Chen, and D. Thanos. 2002. Deciphering the transcriptional histone acetylation code for a human gene. *Cell.* 111:381–392.

Arney, K.L., S. Bao, A.J. Bannister, T. Kouzarides, and A. Surani. 2002. Histone methylation defines epigenetic asymmetry in the mouse zygote. *Int. J. Dev. Biol.* 46:317–320.

Bartl, S., J. Taplick, G. Lagger, H. Khier, K. Kuchler, and C. Seiser. 1997. Identification of mouse histone deacetylase 1 as a growth factor-inducible gene. *Mol. Cell. Biol.* 17:5033–5043.

Chen, H., R.J. Lin, W. Xie, D. Wilpitz, and R.M. Evans. 1999. Regulation of hormone-induced histone hyperacetylation and gene activation via acetylation of an acetylase. *Cell.* 98:675–686.

Clarke, D.J., L.P. O'Neill, and B.M. Turner. 1993. Selective use of H4 acetylation sites in the yeast *Saccharomyces cervisiae*. *Biochem. J.* 294:557–561.

Ekwall, K., T. Olsson, B.M. Turner, G. Cranston, and R.C. Allshire. 1997. Transient inhibition of histone deacetylation alters the structural and functional imprint at fission yeast centromeres. *Cell.* 91:1021–1032.

Fuchimoto, D., A. Mizukoshi, R.M. Schultz, S. Sakai, and F. Aoki. 2001. Post-transcriptional regulation of cyclin A1 and cyclin A2 during mouse oocyte meiotic maturation and preimplantation development. *Biol. Reprod.* 65: 986–993.

Grunstein, M. 1997. Histone acetylation in chromatin structure and transcription. *Nature.* 389:349–352.

Grunstein, M. 1998. Yeast heterochromatin: regulation of its assembly and inheritance by histones. *Cell.* 93:325–328.

Hershkovitz, M., and A.D. Riggs. 1995. Metaphase chromosome analysis by ligation-mediated PCR: heritable chromatin structure and a comparison of active and inactive X chromosome. *Proc. Natl. Acad. Sci. USA.* 92:2379–2383.

Imai, S., C.M. Armstrong, M. Kaeberlein, and L. Guarente. 2000. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature.* 403:795–800.

Jeppesen, P. 1997. Histone acetylation: a possible mechanism for the inheritance of cell memory at mitosis. *Bioessays.* 19:67–74.

Jeppesen, P., and B.M. Turner. 1993. The inactive X chromosome in female mammals is distinguished by a lack of histone H4 acetylation, a cytogenetic marker for gene expression. *Cell.* 74:281–289.

Jenuwein, T., and C.D. Allis. 2001. Translating the histone code. *Science.* 293: 1074–1080.

Kikyo, N., P.A. Wade, D. Guschin, H. Ge, and A.P. Wolffe. 2000. Active remodeling of somatic nuclei in egg cytoplasm by the nucleosomal ATPase ISWI. *Science.* 289:2360–2362.

Kim, J.-M., A. Ogura, M. Nagata, and F. Aoki. 2002. Analysis of the chromatin remodeling in the embryos reconstructed by somatic nuclear transfer. *Biol. Reprod.* 67:760–766.

Kimura, A., T. Umehara, and M. Horikoshi. 2002. Chromosomal gradient of histone acetylation established by Sas2p and Sir2p functions as a shield against gene silencing. *Nat. Genet.* 32:370–377.

Kurdistani, S.K., and M. Grunstein. 2003. Histone acetylation and deacetylation in yeast. *Nat. Rev. Mol. Cell Biol.* 4:276–284.

Kruhlak, M.J., M.J. Hendzel, W. Fischle, N.R. Bertos, S. Hameed, X.-J. Yang, E. Verdin, and D.P. Bazett-Jones. 2001. Regulation of global acetylation in mitosis through loss of histone acetyltransferases and deacetylases from chromatin. *J. Biol. Chem.* 276:38307–38319.

Kuo, M.H., J. Zhou, P. Jambeck, M.E. Churchill, and C.D. Allis. 1998. Histone acetyltransferase activity of yeast Gcn5p is required for the activation of target genes in vivo. *Genes Dev.* 12:627–639.

Martinez-Balbas, M.A., A. Dey, S.K. Rabindran, K. Ozato, and C. Wu. 1995. Displacement of sequence-specific transcription factors from mitotic chromatin. *Cell.* 83:29–38.

Migeon, B.R., S. Luo, M. Jani, and P. Jeppesen. 1994. The severe phenotype of females with tiny ring X chromosomes is associated with inability of these chromosomes to undergo X inactivation. *Am. J. Hum. Genet.* 55:497–504.

Mizzen, C.A., and C.D. Allis. 1998. Linking histone acetylation to transcriptional regulation. *Cell. Mol. Life Sci.* 54:6–20.

Narlikar, G.J., H.Y. Fan, and R.E. Kingston. 2002. Cooperation between complexes that regulate chromatin structure and transcription. *Cell.* 108:475–487.

O'Neill, L.P., and B.M. Turner. 1995. Histone H4 acetylation distinguishes coding regions of the human genome from heterochromatin in a differentiation-dependent but transcription-independent manner. *EMBO J.* 14:3946–3957.

Parekh, B.S., and T. Maniatis. 1999. Virus infection leads to localized hyperacetylation of histones H3 and H4 at the IFN-β promoter. *Mol. Cell.* 3:125–129.

Peterson, C.L., and J.L. Workman. 2000. Promoter targeting and chromatin remodeling by the SWI/SNF complex. *Curr. Opin. Genet. Dev.* 10:187–192.

Rideout, W.M., III, K. Eggan, and R. Jaenisch. 2001. Nuclear cloning and epigenetic reprogramming of the genome. *Science.* 293:1093–1098.

Riggs, A.D., and G.P. Pfeifer. 1992. X-chromosome inactivation and cell memory. *Trends Genet.* 8:169–174.

Roth, S.Y., J.M. Denu, and C.D. Allis. 2001. Histone acetyltransferases. *Annu. Rev. Biochem.* 70:81–120.

Ryan, J., A.J. Llinas, D.A. White, B.M. Turner, and J. Sommerville. 1999. Maternal histone deacetylase is accumulated in the nuclei of *Xenopus* oocytes as protein complexes with potential enzyme activity. *J. Cell Sci.* 112:2441–2452.

Schultz, R.M., W.J. Davis, P. Stein, and P. Svoboda. 1999. Reprogramming of gene expression during preimplantation development. *J. Exp. Zool.* 285: 276–282.

Shestakova, E., M.T. Bandu, J. Doly, and E. Bonnefoy. 2001. Inhibition of histone deacetylation induces constitutive derepression of the β interferon promoter and confers antiviral activity. *J. Virol.* 75:3444–3452.

Smith, C.M., Z.W. Haimberger, C.O. Johnson, A.J. Wolf, P.R. Gafken, Z. Zhang, M.R. Parthun, and D.E. Gottschling. 2002. Heritable chromatin structure: mapping "memory" in histones H3 and H4. *Proc. Natl. Acad. Sci. USA.* 99:16454–16461.

Sobajima, S., F. Aoki, and K. Kohmoto. 1993. Activation of mitogen-activated protein kinase during meiotic maturation in mouse oocytes. *J. Reprod. Fertil.* 97:389–394.

Strahl, B.D., and C.D. Allis. 2000. The language of covalent histone modifications. *Nature.* 403:41–45.

Suka, N., Y. Suka, A.A. Carmen, J. Wu, and M. Grunstein. 2001. Highly specific antibodies determine histone acetylation site usage in yeast heterochromatin and euchromatin. *Mol. Cell.* 8:473–479.

Suka, N., K. Luo, and M. Grunstein. 2002. Sir2p and Sas2p opposingly regulate acetylation of yeast histone H4 lysine 16 and spreading of heterochromatin. *Nat. Genet.* 32:378–383.

Taunton, J., C.A. Hassig, and S.L. Schreiber. 1996. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science.* 272:408–411.

Thorne, A.W., D. Kmiciek, K. Mitchelson, P. Sautiere, and C. Crane-Robinson. 1990. Patterns of histone acetylation. *Eur. J. Biochem.* 193:701–713.

Turner, B.M. 1998. Histone acetylation as an epigenetic determinant of long-term

transcriptional competence. *Cell. Mol. Life Sci.* 54:21–31.

Turner, B.M. 2000. Histone acetylation and an epigenetic code. *Bioessays.* 22:836–845.

Turner, B.M. 2002. Cellular memory and the histone code. *Cell.* 111:285–291.

Turner, B.M., and G. Fellows. 1989. Specific antibodies reveal ordered and cell-cycle related use of histone-H4 acetylation sites in mammalian cells. *Eur. J. Biochem.* 179:131–139.

Vogelauer, M., L. Rubbi, I. Lucas, B.J. Brewer, and M. Grunstein. 2002. Histone acetylation regulates the time of replication origin firing. *Mol. Cell.* 10: 1223–1233.

Wakayama, T., A.C. Perry, M. Zuccotti, K.R. Johnson, and R. Yanagimachi. 1998. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature.* 394:369–374.

Weintraub, H. 1985. Assembly and propagation of repressed and depressed chromosomal states. *Cell.* 42:705–711.

Wilmut, I., A.E. Schnieke, J. McWhir, A.J. Kind, and K.H.S. Campbell. 1997. Viable offspring derived from fetal and adult mammalian cells. *Nature.* 385: 810–813.

Whitten, W.K. 1971. Nutrient requirement for the culture of preimplantation embryos. *Adv. Biosci.* 6:129–139.

Yoshida, M., M. Kijima, M. Akita, and T. Beppu. 1990. Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. *J. Biol. Chem.* 265:17174–17179.

Zhang, J., F. Xu, T. Hashimshony, I. Keshet, and H. Cedar. 2002. Establishment of transcriptional competence in early and late S phase. *Nature.* 420:198–202.