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—Full Paper—

Dynamic Changes in Histone Acetylation During Sheep Oocyte Maturation

Lian-Sheng TANG[1)], Qiang WANG[2)], Bo XIONG[2)], Yi HOU[2)], Yong-Zhong ZHANG[2)], Qing-Yuan SUN[2)] and Shu-Ying WANG[1)]

[1)]College of Animal Science and Veterinary Medicine, Shandong Agricultural University, Taian, Shandong 271018 and [2)]State Key Laboratory of Reproduction Biology, Institute of Zoology, Chinese Academy of Science, Beijing 100080, P.R. China

Abstract. The changes in histone acetylation are not always consistent in various cell types and at different developmental stages. We immunostained specific antibodies against acetylated lysine 9 of histone H3 and acetylated lysines 5 and 12 of histone H4 in an effort to understand the detailed changes in histone acetylation during sheep oocyte meiosis. We found that the acetylation fluorescence signals of H3/K9 and H4/K12 on chromatin appeared intensively in the germinal vesicle (GV), late-GV (L-GV), and germinal vesicle breakdown (GVBD) stages and became weak in metaphase I (MI); however staining reappeared in anaphase I-telophase-I (AI-TI) and metaphase II (MII). Furthermore, staining was detected in the first polar bodies. The fluorescence signals of H4/K5 first appeared in the MI stage and became intensive in the AI-TI stage; however they were barely detectable in MII stage chromosomes and first polar bodies. We conclude that the acetylation patterns of H3/K9 and H4/K12 during oocyte meiotic maturation are similar and that the pattern of H4/K5 is unique.

Key words: Histone acetylation, Meiotic maturation, Sheep oocyte, Trichostain A

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The basic structural unit of eukaryotic chromosomes is a DNA protein complex called the nucleosome that consists of 147 base pairs of DNA wrapped around an octamer of the H2A, H2B, H3, and H4 histone proteins. The nucleosome histones are thought to play important roles in various cellular functions. It is well known that post-translational acetylation, methylation, phosphorylation, and ubiquitination of histones play an intrinsic role in transcription regulation [1, 2]. In these post-translational modifications, histone acetylation affects chromation conformation [3], correlates with gene activity [4, 5], and is required for orderly meiosis [6]. The N-terminal tails of histone H3 and H4 have a critical role in the folding of higher order chromatin structure [7–9]. The different effects of histone acetylaion on chromatin organization have been analyzed both *in vitro* and *in vivo* [10, 11] and in mitosis and in meiosis [12]. Recent results show that the acetylation of different lysines of histone is associated with a diversity of chromatin-related processes in mitosis [13] and is necessary for orderly meiosis [6]. The lysine residue-specific changes in histone acetylation during pig oocyte meiosis have distinctive characteristics [6, 15] compared with those of mouse oocytes [14].

In the present study, we investigated the changes in acetylation of lysine K9 of histone H3, and lysine K5, K12 of histone H4 in sheep oocytes at different stages of maturation.

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Correspondence: S.Y. Wang (e-mail: sywang@sdau.edu.cn)

Materials and Methods

All chemicals used in this study were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) except for those specifically mentioned.

Rabbit Antibodies polyclonal anti-acetyl lysine 5 of histone H4 (AcH4/K5) antibody and anti-acetyl lysine 12 of histone H4 (AcH4/K12) antibody were purchased from Upstate Biotechnology (Lake Placid, NY, USA). Anti-acetyl lysine 9 of histone H3 (AcH3/K9) antibody was purchased from Cell Signaling Technology (Beverly, MA, USA).

Oocyte collection and culture

Ovaries were collected from sheep and transported to the laboratory at room temperature within 2–3 h in D-PBS (Dulbecco's PBS) with antibiotics. After three washes with penicillin and streptomycin in fresh PBS the ovaries were sliced using a razor blade in sterile Petri dishes containing Hepes-buffered TCM-199 supplemented with 0.1% (w/v) polyvinyl alcohol (PVA). After washing three times with maturation medium (see below), the oocytes with a compact cumulus mass and evenly granulated ooplasm were selected on the basis of their dimensions using the calibrated eyepiece of an inverted microscope for maturation culture. The medium used for maturation culture was TCM-199 (Gibco, Grand Island, NY, USA) supplemented with 75 μg/ml penicillin, 50 μg/ml streptomycin 10% FBS (Gibco), 0.5 μg/ml FSH, 0.5 μg/ml LH, and 0.1 μg/ml E_2 [16]. Groups of 20 oocytes were cultured in 100 μl drops of maturation medium at 38.8 C in a humidified environment of 5% CO_2 in air. The cumulus-oocyte complexes were cultured for different periods of time, and then the cumulus cells were removed by treatment with 300 IU/ml hyaluronidase in TCM-199. Cumulus-free oocytes were used in the immunofluorescent experiments.

Trichostatin A (TSA) treatment

TSA , a general inhibitor of histone deacetylation (HDAC), solution prepared in DMSO was diluted in maturation medium to yield a final concentration of 100 ng/ml. Some of the fully grown oocytes were treated with TSA for 2 h in the GV stage. The rest of the fully grown oocytes were cultured for 4 h in maturation medium and subsequently treated with TSA for 4 h. Finally, the cumulus-oocyte complexes were denuded as described above, and the cumulus-free oocytes were used in the following experiments.

Immunofluorescent staining and confocal microscopy

After removing the zona pellucida in acidic Tyrode's medium (pH 2.5), the oocytes were fixed with 4% paraformaldehyde (pH 7.4) in D-PBS for at least 1 h at room temperature or overnight at 4 C. They were then permeabilized with 1% Triton X-100 for 4 h at 37 C, washed three times with 1% BSA-supplemented D-PBS (blocking solution), and then blocked in blocking solution for 1.5 h at room temperature. The oocytes were subsequently incubated with primary antibodies diluted to 1:200 overnight at 4 C. After washing three times in PBS containing 0.1% Tween 20 and 0.01% Triton X-100 (washing solution) for 5 min each, the antibodies that bound to the oocytes were probed with FITC-conjugated anti-rabbit IgG diluted to 1:100 for 1 h at room temperature. They were then washed extensively before their DNA was stained for 10 min with 10 μg/ml PI (propidium iodide). Following extensive washing, the oocytes were mounted on glass slides supported by four columns of a mixture of petroleum jelly and paraffin (9:1) and were covered with a coverslip. The samples were observed under a confocal laser scanning microscope (LSM 510 META; Carl Zeiss, Oberkochen, Germany) as soon as possible. Each experiment was repeated three times, and at least 30 oocytes were examined at each stage each time.

Evaluation of unclear status

Denuded oocytes were mounted onto slides, fixed in acetic acid: ethanol [1:3 (v/v)] for at least 48 h, stained with 1% orcein, and examined with a phasecontrast microscope.

Results

Nuclear maturation of sheep oocytes during in vitro culture

As shown in Fig. 1, 93.2% of the oocytes were at the germinal vesicle (GV) stage at 0 h of culture, 84.3% of them underwent germinal vesicle breakdown (GVBD) by 8 h of culture, and 67.5% of them proceeded to the metaphase I (MI) by 12 h of culture. When the oocytes were examined after 16 h, 70.6% of them were at anaphase and telophase I (AI-TI). The proportion of oocytes at the MII stage

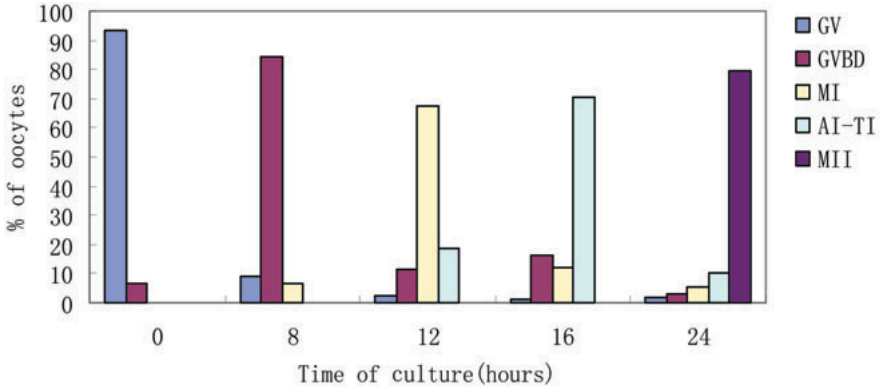
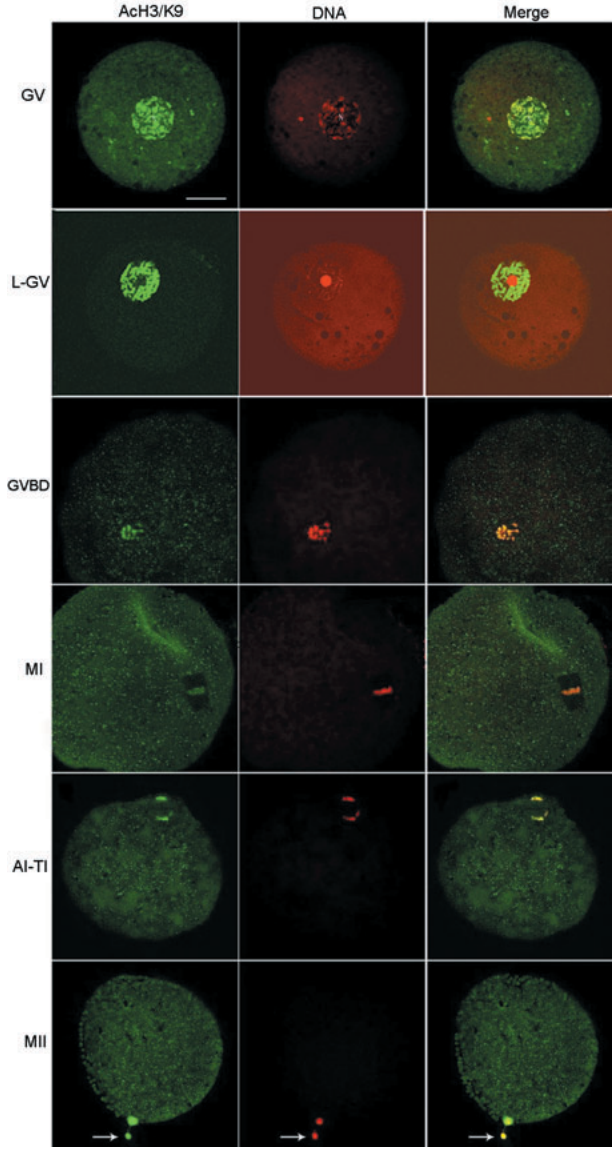


Fig 1. Nuclear maturation of sheep oocytes during *in vitro* culture. GV, germinal vesicle; GVBD, germinal vesicle breakdown stage; MI, metaphase I; AI-TI, anaphase and telophase I; MII, metaphase II. The same abbreviations were used in the following figures.



was 79.4% after 24 h of culture. This data was obtained by orcein staining and was a critical prerequisite for the following experimental design.

H3/K9 acetylation changes during sheep oocyte meiotic maturation

Sheep oocytes at different stages were immunolabeled with the specific antibody against acetylated lysine 9 of histone H3. The results are shown in Fig. 2. At the GV and L-GV stages, intense fluorescence signals were detected for anti-acetyl lysine 9 of histone H3 around the GV stage nucleolus. As the oocytes underwent GVBD and progressed into the MI stage, the fluorescence signals reduced. Fluorescence signals were detected on the chromosomes and intense signals of H3/K9 were observed in the first polar body (Fig. 2, arrow) as the oocytes progressing into AI-TI or even MII stage.

H4/K12 acetylation changes during sheep oocyte meiotic maturation

Sheep oocytes were immunolabeled at different stages with the specific antibody against acetylated lysine 12 of histone H4. The results are shown in Fig. 3. The H4/K12 acetylation pattern was similar

Fig 2. Acetylation changes of H3/K9 during meiotic maturation of sheep oocytes. Oocytes at distinct stages of meiosis were immunostained with the anti-acetyl histone (H3/K9) antibody. L-GV: oocytes at late germinal vesicle stage. Arrows indicate first polar bodies. DNA is stained with PI and is shown in red. The scale bar represents 30 μm.

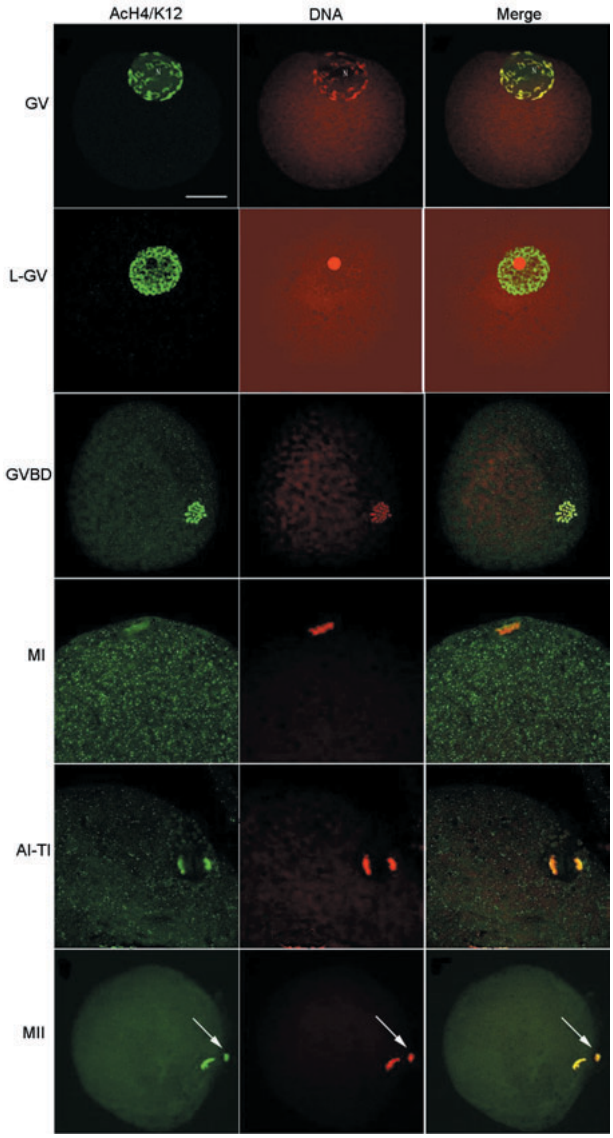


Fig 3.

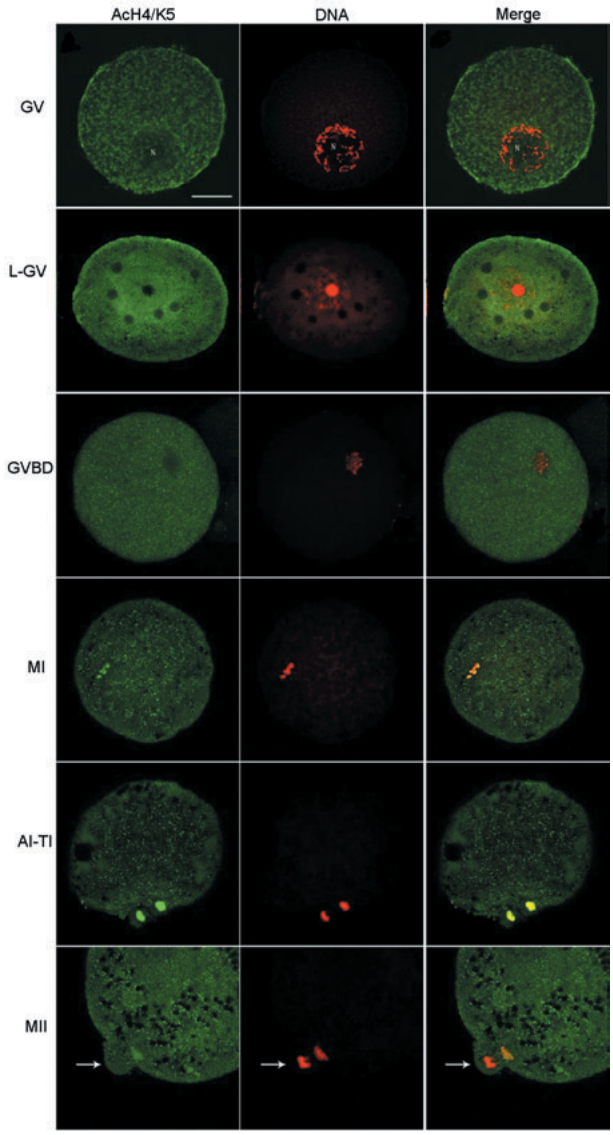


Fig 4.

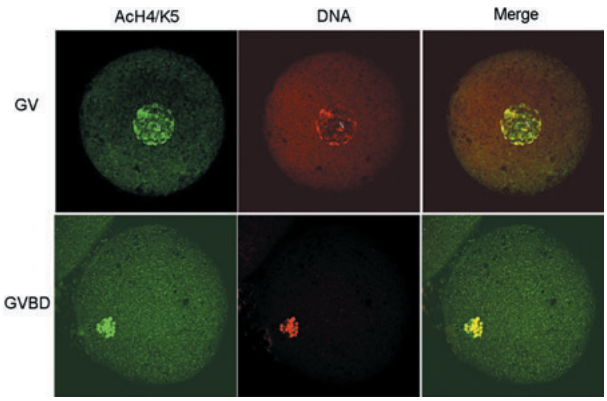


Fig 5.

Fig 3. Acetylation changes of H4/K12 during meiotic maturation of sheep oocytes. Oocytes at distinct stages of meiosis were immunostained with the anti-acetyl histone (H4/K12) antibody. The scale bar represents 30 μm.

Fig 4. Acetylation changes of H4/K5 during meiotic maturation of sheep oocytes. Oocytes at distinct stages of meiosis were immunostained with anti-acetyl histone (H4/K5) antibody. The scale bar represents 30 μm.

Fig 5. AcH4/K5 immunofluorescence induced by treatment with 100 ng/ml TSA in the GV and GVBD stages. The scale bar represents 30 μm.

to that of H3/K9 during sheep oocyte meiotic maturation. At the GV stage, the signals for anti-acetyl lysine 12 of histone H4 were observed around the nucleolus. The oocytes progressed into the L-GV stage and the intense fluorescence signals of the anti-acetyl lysine 12 of histone H4 were detected when nuclear membranes were less distinct and the chromatins were observed to be an irregular network. However, the intensity of the signals reduced from the L-GV to MI stage. The signals of acetylation were stronger at the GVBD stage than at the MI stage. The fluorescence signals for immunostaining of the anti-acetyl lysine 12 of histone H4 increased again during the AI-TI stage. Furthermore, intense signals were simultaneously detected on MII stage chromosomes and first polar bodies (Fig. 3, arrow).

H4/K5 acetylation changes during sheep oocyte meiotic maturation

Sheep oocytes at different stages were immunolabeled with the specific antibody against acetylated lysine 5 of histone H4. The results are shown in Fig. 4. H4/K5 showed a different acetylation pattern from those of H3/K9 and H4/K12. No fluorescence signals were detected for anti-acetyl lysine 5 of histone H4 in the GV, L-GV, and GVBD stages. However, hyperacetylation of H4/K5 was efficiently induced by the trichostain A (TSA) treatment (Fig. 5) [17]. These results indicate that oocytes hyperacetylated at H4/K5 by TSA treatment undergo GVBD. At MI, weak signals were observed for anti-acetyl lysine 5 of histone H4. As the oocytes progressed into the AI-TI stage, the fluorescence signals for anti-acetyl lysine 5 of histone H4 were strong. The signals decreased dramatically and disappeared in the first polar bodies as the oocytes progressed into MII stage (Fig. 4, arrow).

Discussion

The results of this work represent the residue-specific acetylation changes in H3/K9, H4/K5, and H4/K12 during meiotic maturation. Histone acetylation is a reversible process, and histone deacetylation is responsible for the opposite reaction. They are both determined by the combined activities of two enzyme families, histone acetyltransferaes (HATs) and deacetylases (HDACs) [18–20].

Histone acetylation plays important roles in meiosis [21], and histone deacetylation plays key roles in chromatin-based mechanisms [22]. We divided oocyte maturation into six stages from GV to MII to describe the detailed dynamic changes in the acetylation of H3/K9, H4/K5, and H4/K12. From the GV to L-GV stage, fluorescence signals were present for acetylation H3/K9 and H4/K12 (Figs. 2, 3), but the signals for H4/K5 were absent (Fig. 4). However the fluorescence signal for acetylated H4/K5 was detected after TSA treatment (Fig. 5). These results indicate that H3/K9 and H4/K12 acetylation may be required for transition from the GV to L-GV stage. Based on our results, H4/K5 deacetylation may not be required for progression through these stages, since TSA-treated oocytes were able to undergo GVBD with acetylated H4/K5. In pig [6, 15, 23] and mouse [13] oocytes, acetylation of H4/K5 is obvious from the GV to L-GV stage. This discrepancy may be associated with a species difference. Full chromatin condensation was achieved in the GVBD stage (Figs. 2–4). Magnaghi-Jaulin and Jaulin [24] reported that HDAC activity is required to establish chromosome condensation. This corresponds with the facts that H4/K5 histone acetylation did not persist in the GVBD stage (Fig. 4) and that the fluorescence signals of H3/K9 histone acetylation weakened with progression from the GV to GVBD stage (Fig. 2) in this study. On the other hand, chromosome condensation of sheep oocytes may not require H4/K5 HDAC, as shown in the TSA experiment.

During meiotic maturation after GVDB, the condensed chromosomes of the oocytes were gathered by the meiotic spindle and aligned on the metaphase plate (Figs. 2–4) [16]. The chromosomes were then separated into two groups, indicating the occurrence of anaphase and telophase I (AI-TI; Figs. 2–4). This was followed by extrusion of first polar bodies and progression into meiosis II (MII; Figs. 2–4). Histone deacetylation plays a major role in order to "clean" the chromatin [25]. This caused H3/K9 and H4/K12 acetylation to weaken (Figs. 2, 3) when the oocytes proceeded from the GVBD to MI stage. However, strong acetylation signals and first polar bodies were detected again between the AI-TI and MII stages (Figs. 2–4). These results are in accordance with the report of Wang *et al*. [6], which showed that highly acetylated chromatin is

observed at the AI-TI stage in pig oocytes; similar results have also been reported for meiosis [26]. H3/K9 and H4/K12 acetylation may be necessary to enable oocytes to enter the MII stage after they extrude the first polar bodies (Figs. 2, 3), although previous studies have shown no signals for anti-Ac-H4/K12 or anti-Ac-H3/K14 in MII stage mouse oocytes [14]. Interestingly, H4/K5 histone acetylation first appeared in the MI stage, tended to peak in the AI-TI stage, and became weak or barely detectable in the at MII stage (Fig. 4). However, previous studies have shown acetylation of H4/K5 in AT-TI stage mouse oocytes [14] and L-GVBD, MI stages in pig oocytes [6, 15]. This may be due to differences between sheep, mouse, and pig oocytes.

In contrast to the changes in the acetylation patterns of Xenopus, porcine, and mouse oocytes, there are distinctive changes in the acetylation of H3/K9, H4/K5, and H4/K12 during meiotic maturation of sheep oocytes. Taken together, this data indicates that there is a difference in the histone acetylation patterns of the oocytes of some species and that the histone acetylation level and chromatin structure do not always correlate.

In conclusion, H3/K9 and H4/K12 have similar fluctuation trends during ovine oocyte maturation, with staining from GV to L-GV stages, decreased staining from the GVBD to MI stages, and elevated staining again from the AI-TI to MII stages. H4/K5 has a unique acetylation pattern. It had strong acetylation staining during the AI-TI stage and decreased acetylation staining during the MII stage.

Acknowledgments

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References

1. **Felsenfeld G, Groudine M**. Controlling the double helix. *Nature* 2003; 421: 448–453.
2. **Turner BM**. Cellular memory and the histone code. *Cell* 2002; 111: 285–291.
3. **Cheung WL, Briggs SD, Allis CD**. Acetylation and chromosomal functions. *Curr Opin Cell Biol* 2000; 12: 326–333.
4. **Schubeler D, MacAlpine DM, Scalzo D, Wirbelauer C, Kooperberg C, van Leeuwen F, Gottschling DE, O'Neill LP, Turner BM, Delrow J, Bell SP, Groudine M**. The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. *Genes Dev* 2004; 18: 1263–1271.
5. **Verdone L, Caserta M, Di Mauro E**. Role of histone acetylation in the control of gene expression. *Biochem Cell Biol* 2005; 83: 344–353.
6. **Wang Q, Yin S, Ai JS, Liang CG, Hou Y, Chen DY, Schatten H, Sun QY**. Histone deacetylation is required for orderly meiosis. *Cell Cycle* 2006; 5: 766–774.
7. **Grunstein M**. Histone acetylation in chromatin structure and transcription. *Nature* 1997; 389: 349–352.
8. **Moore SC, Ausio J**. Major role of the histones H3–H4 in the folding of the chromatin fiber. *Biochem Biophys Res Commun* 1997; 230: 136–139.
9. **Simpson RT**. Structure of the chromatosome, a chromatin particle containing 160 base pairs of DNA and all the histones. *Biochemistry* 1978; 17: 5524–5531.
10. **Gavazzo P, Vergani L, Mascetti GC, Nicolini C**. Effects of histone acetylation on chromatin structure. *J Cell Biochem* 1997; 64: 466–475.
11. **Riggs MG, Whittaker RG, Neumann JR, Ingram VM**. n-Butyrate causes histone modification in HeLa and Friend erythroleukaemia cells. *Nature* 1977; 268: 462–464.
12. **Li Y, Butenko Y, Grafi G**. Histone deacetylation is required for progression through mitosis in tobacco cells. *Plant J* 2005; 41: 346–352.
13. **Cimini D, Mattiuzzo M, Torosantucci L, Degrassi F**. Histone hyperacetylation in mitosis prevents sister chromatid separation and produces chromosome segregation defects. *Mol Biol Cell* 2003; 14: 3821–3833.
14. **Kim JM, Liu H, Tazaki M, Nagata M, Aoki F**. Changes in histone acetylation during mouse oocyte meiosis. *J Cell Biol* 2003; 162: 37–46.
15. **Endo T, Naito K, Aoki F, Kume S, Tojo H**. Changes in histone modifications during *in vitro* maturation of porcine oocytes. *Mol Reprod Dev* 2005; 71: 123–128.
16. **Fukui Y, Glew AM, Gandolfi F, Moor RM**. *In vitro* culture of sheep oocytes matured and fertilized *in vitro*. *Theriogenology* 1988; 29: 883–891.
17. **Yoshida M, Kijima M, Akita M, Beppu T**. Potent

and specific inhibition of mammalian histone deacetylase both *in vivo* and *in vitro* by trichostatin A. *J Biol Chem* 1990; 265: 17174–17179.

18. **Bertos NR, Wang AH, Yang XJ**. Class II histone deacetylases: structure, function, and regulation. *Biochem Cell Biol* 2001; 79: 243–252.
19. **Grant PA, Berger SL**. Histone acetyltransferase complexes. *Semin Cell Dev Biol* 1999; 10: 169–177.
20. **Kuo MH, Allis CD**. Roles of histone acetyltransferases and deacetylases in gene regulation. *Bioessays* 1998; 20: 615–626.
21. **Akiyama T, Kim JM, Nagata M, Aoki F**. Regulation of histone acetylation during meiotic maturation in mouse oocytes. *Mol Reprod Dev* 2004; 69: 222–227.
22. **Swedlow JR, Hirano T**. The making of the mitotic chromosome: modern insights into classical questions. *Mol Cell* 2003; 11: 557–569.
23. **Endo T, Naito K, Kume S, Nishimura Y, Kashima K, Tojo H**. Activities of maturation-promoting factor (MPF) and mitogen-activated protein kinase (MAPK) are not required for the global histone deacetylation observed after germinal vesicle breakdown (GVBD) in porcine oocytes. *Reproduction* 2006; 131: 439–447.
24. **Magnaghi-Jaulin L, Jaulin C**. Histone deacetylase activity is necessary for chromosome condensation during meiotic maturation in *Xenopus* laevis. *Chromosome Res* 2006; 14: 319–332.
25. **Smillie DA, Llinas AJ, Ryan JT, Kemp GD, Sommerville J**. Nuclear import and activity of histone deacetylase in *Xenopus* oocytes is regulated by phosphorylation. *J Cell Sci* 2004; 117: 1857–1866.
26. **Kruhlak MJ, Hendzel MJ, Fischle W, Bertos NR, Hameed S, Yang XJ, Verdin E, Bazett-Jones DP**. Regulation of global acetylation in mitosis through loss of histone acetyltransferases and deacetylases from chromatin. *J Biol Chem* 2001; 276: 38307–38319.