Alterations in epigenetic modifications during oocyte growth in mice

Shun-ichiro Kageyama¹, Honglin Liu¹,², Naoto Kaneko¹, Masatoshi Ooga¹, Masao Nagata¹ and Fugaku Aoki¹

¹Department of Integrated Biosciences, Graduate School of Frontier Sciences, University of Tokyo, Room #302, Seimei-Building, Kashiwa, Chiba 277-8571, Japan and ²College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, Japan

Correspondence should be addressed to F Aoki; Email: aokif@k.u-tokyo.ac.jp

Abstract

During oocyte growth, chromatin structure is altered globally and gene expression is silenced. To investigate the involvement of epigenetic modifications in the regulation of these phenomena, changes in global DNA methylation and in various histone modifications, i.e. acetylation of H3K9, H3K18, H4K5, and H4K12, and methylation of H3K4 and H3K9, were examined during the growth of mouse oocytes. Immunocytochemical analysis revealed that the signal intensities of all these modifications increased during growth and that fully grown, germinal vesicle (GV)-stage oocytes showed the most modifications. Since acetylation of most of the lysine residues on histones and methylation of H3K4 are associated with active gene expression, the increased levels of these modifications do not seem to be associated with gene silencing in GV-stage oocytes. Given that there are two types of GV-stage oocytes with different chromatin configurations and transcriptional activities, the epigenetic modification statuses of these two types were compared. The levels of all the epigenetic modifications examined were higher in the SN(surrounded nucleolus)-type oocytes, in which highly condensed chromatin is concentrated in the area around the nucleolus and gene expression is silenced than in the NSN(not surrounded nucleolus)-type oocytes, in which less-condensed chromatin does not surround the nucleolus and gene expression is active. In addition, the expression levels of various enzymes that catalyze histone modifications were shown by RT-PCR to increase with oocyte growth. Taken together, the results show that all of the epigenetic modifications increased in a concerted manner during oocyte growth, and suggest that these increases are not associated with gene expression.

Introduction

Generation of gametes during oogenesis is a crucial process in the creation of new life for the next generation. Oocytes that are arrested at the prophase of the first meiosis grow and increase in size in the ovaries of female mouse pups after birth, and these oocytes reach the germinal vesicle (GV) stage with maximum size at around 3 weeks after birth. These growing oocytes express several oocyte-specific genes, such as c-mos and zp3. However, as they approach the GV stage, transcription stops and remains silent until after the oocytes are fertilized. In mice, transcription starts at mid S/G2 phase in the one-cell stage, when oocyte-specific genes are not transcribed, while some genes not previously expressed in oocytes are transcribed (Schultz 1993, Aoki et al. 1997, Kigami et al. 2003). Therefore, the gene expression pattern is altered globally so as to transform the differentiated oocytes into totipotent embryos, which suggests that global genome remodeling occurs during these periods.

There is evidence to support the idea that chromatin structure is altered globally during oocyte growth. Mouse oocytes isolated from antral follicles show two essentially different chromatin configurations (Debey et al. 1993, Zuccotti et al. 1995). These are termed SN, in which the chromatin is highly condensed and is concentrated in the area around the nucleolus and gene expression is silenced than in the NSN(not surrounded nucleolus)-type oocytes, in which less-condensed chromatin does not surround the nucleolus and gene expression is active. In addition, the expression levels of various enzymes that catalyze histone modifications were shown by RT-PCR to increase with oocyte growth. Taken together, the results show that all of the epigenetic modifications increased in a concerted manner during oocyte growth, and suggest that these increases are not associated with gene expression.
Growing oocytes. The SN-type configuration is strictly correlated with the cessation of transcription. In contrast, transcriptional activity is detected in NSN-type oocytes of any age (Bouniol-Baly et al. 1999, De La Fuente & Eppig 2001, Liu & Aoki 2002). Therefore, the change in the chromatin configuration is likely to play an essential role in the alteration of gene expression during oocyte growth and seems to be involved in global genome remodeling, although the mechanism underlying this change remains to be elucidated.

Recent studies have revealed that epigenetic modifications, such as DNA methylation and histone modifications, play important roles in the regulation of chromatin structure and gene expression (Robertson & Wolff 2000, Jenuwein & Allis 2001, Reik et al. 2001). When cells change their characteristics, e.g. during differentiation orubbolulation, genome-wide alterations in these modifications occur. Indeed, genome-wide alterations of epigenetic modifications have been reported in both early and late oogenesis. At around E8.0, germ cells concomitantly and significantly reduce both di-methylation of lysine 9 on histone H3 (H3K9me2) and DNA methylation (Seki et al. 2005). Furthermore, although all the N-terminal lysine residues of the histones in the nucleus are acetylated in GV-stage oocytes, they are prominently deacetylated after germinal vesicle breakdown (Kim et al. 2003, Endo et al. 2005). Therefore, genome-wide alterations of DNA and histone modifications may also be involved in genome remodeling during oocyte growth. However, little is known about these modifications at this stage. In the present study, using immunocytochemistry, we conducted a comprehensive analysis of the genome-wide alterations of epigenetic modifications during oocyte growth in mice. In this analysis, the involvement of epigenetic modifications in the differentiation of chromatin configuration, i.e. NSN- to SN-type, was also examined. In addition, we examined the expression of the enzymes that catalyze histone modifications in growing oocytes.

**Materials and Methods**

**Collection of oocytes**

Growing oocytes were collected from the ovaries of 5-, 10-, and 15-day-old female BDF1 mice (SLC, Shizuoka, Japan), as described below. Ovaries were removed with fine scissors and freed from the surrounding tissues with a 27G needle under the operation microscope. Each ovary was placed in a 200 µl drop PBS, transferred to 0.5% trypsin–EDTA (Gibco-BRL), and incubated at 38 °C with agitation. After 10 min, the ovary was washed with Whitten’s medium (Whitten 1971), and oocytes with diameters of 30–40, 40–50, and 50–65 µm for the 5-, 10-, and 15-day-old mice respectively, which were regarded as being of normal size for their respective stages of development, were collected (Bao et al. 2000, Kageyama et al. 2005).

Forty-six hours after injection of 5 IU of pregnant mare’s serum gonadotropin (Sankyo Co., Ltd., Tokyo, Japan), GV-stage oocytes were collected from 4-week-old BDF1 mice (SLC) by puncturing the follicles with a sharp needle. The oocytes were placed in Whitten’s medium that was supplemented with 20 mM HEPES and 0.2 mM 3-isobutyl-1-methylxanthine. The oocytes were liberated from the surrounding cumulus cells by gentle pipetting through a narrow-bore glass pipette. Only those oocytes with diameter >70 µm were used as GV-stage oocytes.

All procedures described here were reviewed and approved by the University of Tokyo Institutional Animal Care and Use Committee and were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals.

**Immunofluorescence confocal microscopy**

Oocytes were fixed for 1 h with 3.7% paraformaldehyde in PBS. After washing with PBS/0.1% BSA, the oocytes were permeabilized with 0.5% Triton X-100 in PBS for 15 min at room temperature and then immunostained with antibodies against acetylated lysines 5 and 12 of histone H4 (Upstate Biotechnology, Charlottesville, VA, USA), lysines 9 (Cell Signaling Technology Inc., Beverly, MA, USA) and 18 (Upstate Biotechnology) of histone H3, di-methylated lysine 4 (Upstate Biotechnology) and tri-methylated lysine 4 (Abcam, Cambridge, MA, USA) of histone H3, di-methylated lysine 9 (Upstate Biotechnology) and tri-methylated lysine 9 (Abcam) of histone H3, and 5-methylated cytosine (Eurogentec, Seraing, Belgium). To detect 5-methyl-cytosine (5-MeC), the cells were pretreated with 2 N HCl at room temperature for 30 min and then neutralized for 20 min with PBS/0.1% PBS, before treatment with the antibody. Treatment with the primary antibodies (1:100 dilutions) was performed at 4 °C overnight. The cells were incubated with fluorescein (FITC)-conjugated anti-rabbit or anti-mouse secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA, USA; 1:50 dilution) at room temperature for 45 min. To visualize the DNA, the cells were counterstained with propidium iodide. The cells were mounted on glass slides with VectaShield (Vector Laboratories, Burlingame, CA, USA) and observed under the Carl Zeiss 510 laser-scanning confocal microscope (Carl Zeiss Microlmaging GmbH, Oberkochen, Germany). The oocytes and embryos at all stages were subjected to immunocytochemistry and mounted on glass slides.

Fluorescence was detected using a laser-scanning confocal microscope (LSM501, Carl Zeiss, Tokyo). For detection, the laser power was set to the level at which the oocyte with the strongest fluorescence intensity showed an almost saturated signal in order to adjust the
signal intensity such that the signals of all of the oocytes were below the saturated level. Semi-quantitative analysis of the fluorescence intensities from the images obtained by laser-scanning confocal microscopy was conducted using the NIH Image program (National Institutes of Health, Bethesda, MD, USA). The pixel value/unit area was measured for the nucleus, and the average value for two different regions of the cytoplasm was subtracted as background. This value was multiplied by the thickness of nucleus to correct for differences in nucleus size between oocytes. Nuclear thickness was measured by confocal microscopy. In each experiment, the average value calculated for the nuclei of GV-stage oocytes was set at 100% and the values for the growing oocytes were expressed relative to this value. In comparisons with the SN and NSN types, the average value for the SN-type oocytes was set at 100%.

**RT-PCR**

Total RNA samples were isolated from oocytes and embryos using ISOGEN (Nippon Gene, Tokyo, Japan), as described previously (Kageyama et al. 2004). The RNA was reverse-transcribed in a 20 µl reaction mixture that contained 5 U ReverScript II (Wako, Osaka, Japan) and 0.5 µg oligo(dT)12–18 primer (Invitrogen Corp.) at 42 °C for 1 h, followed by 51 °C for 30 min. The template mRNA was digested with 60 U RNase H (TaKaRa, Shiga, Japan) at 37 °C for 20 min. As the external control, 50 pg of rabbit α-globin RNA was added to each tube before the isolation of total RNA.

PCR was performed using the iCycler (Bio-Rad). The reaction mixture consisted of template cDNA that was derived from four oocytes or embryos, 0.2 µM of each primer, 300 µM dNTPs, 3 mM MgCl2, and 0.05 U/µl ExTaq DNA polymerase (TaKaRa). The sequences of the PCR primers used are shown in Supplemental Table S1. PCR was performed for 32 cycles for rabbit α-globin, ESET, and Smyd3, and for 40 cycles for the other genes. Each cycle consisted of denaturation at 95 °C for 15 s, annealing for 15 s, and extension at 72 °C for 20 s. The PCR products were separated by electrophoresis in a 2% agarose gel and stained with ethidium bromide. The gel image was obtained using the DT-20MP u.v. illuminator (ATTO, Tokyo, Japan) and the relative amounts of the PCR products were determined by measuring the densities of the bands using the NIH image software. The values for the transcripts of the target genes were normalized with those for the rabbit α-globin mRNA.

**Results**

**Changes in histone acetylation during oocyte growth**

Global alterations in histone acetylation during oocyte growth were examined by immunocytochemistry. The acetylation levels at K9 and K18 on histone H3 (H3K9ac and H3K18ac respectively), and at K5 and K12 on histone H4 (H4K5ac and H4K12ac respectively) were examined in oocytes collected from 5-, 10-, and 15-day-old mice, and in GV-stage oocytes (Fig. 1). The results of fluorescence quantification showed that the levels of acetylation in any of these lysine residues increased with the age of the mice or the growth of the oocytes. The patterns of increased acetylation levels were similar for all the residues, with the sole exception of H4K5ac (Fig. 2). In these residues, the acetylation levels were low in oocytes from 5-day-old mice and were slightly increased in oocytes from 10-day-old mice. The acetylation levels increased dramatically in 15-day-old mice and increased further in GV-stage oocytes. Therefore, the acetylation levels of these lysine residues were inversely correlated with transcriptional activity. In a previous report, using a reporter gene, active transcription was detected when the plasmid was microinjected into oocytes with diameters < 50 µm, which correspond to the 5- and 10-day-old oocytes in the present study, while transcription was much reduced in oocytes with diameters > 50 µm, which correspond to the 15-day-old and GV-stage oocytes used in the present study (Worrad et al. 1994). In the case of H4K5, the acetylation level increased slightly up to day 15, and subsequently increased dramatically in the GV-stage oocytes.

The di- and tri-methylation of H3K4 (H3K4me2 and H3K4me3 respectively), and H3K9 (H3K9me2 and H3K9me3 respectively) were examined in the growing oocytes (Fig. 3). It is known that methylation of H3K4 and H3K9 is involved in the activation and suppression of gene expression respectively (Jenuwein & Allis 2001, Grewal & Moazed 2003). The levels of H3K4me2, H3K4me3, and H3K9me2 increased slightly on day 10 and then increased significantly on day 15 (P<0.0001, by Student’s t-test). In the GV-stage oocytes, these methylation levels increased further (Fig. 4). However, H3K9me3 remained at a low level until day 15 or slightly increased on day 15, and then increased prominently in the GV-stage oocytes.

In the nuclei of oocytes from mice of any age, with the exception of GV-stage oocytes, dense staining for H3K4me3 and H3K9me3 was detected (Fig. 3). These signals co-localized with those for the DNA, with intense fluorescence that presumably represents heterochromatin regions, since the dotted signals for tri-methylated lysines were completely superimposed on those for DNA in the merged images (Supplemental Figs S1 and S2). In the GV-stage oocytes, the fluorescence signals for tri-methylated lysines co-localized uniformly with the DNA signals. On the other hand, in oocytes at any stage, the signals for di-methylated lysines co-localized uniformly with the DNA signals and few of the dotted
signals could be superimposed on those for DNA with intense fluorescence (Supplemental Figs S1 and S2).

**Changes in DNA methylation during oocyte growth**

The level of 5-MeC increased with oocyte growth, with a slight increase on day 10 and marked increases thereafter, until the GV stage (Fig. 5). As observed for trimethylated H3K4 and H3K9, several of the dotted signals for 5-MeC could be superimposed on those for DNA in oocytes from all stages, with the exception of the GV stage (Fig. 5A and Supplemental Fig. S3). In the case of the GV-stage oocytes, the 5-MeC signal was spread over the entire region in which DNA was detected.

**Different epigenetic modifications in two types of GV-stage oocytes with different chromatin configurations**

SN- and NSN-type oocytes were distinguished according to the chromatin configurations deduced from images of DNA-stained, GV-stage oocytes. The levels of the various epigenetic modifications, i.e. H4K5 and H4K12 acetylation, H3K9 methylation, and 5-MeC, were compared between these two oocyte types (Fig. 6A). Quantification of the immunofluorescence signals revealed that the levels of all these modifications were higher in SN-type oocytes than in NSN-type oocytes (Fig. 6B). These differences did not come from the different patterns of chromatin condensation, which could cause the

---

**Figure 1** Alterations in global histone acetylation during oocyte growth. Growing oocytes, collected from 5-, 10-, and 15-day-old mice, and GV-stage oocytes were immunostained with antibodies against acetylated lysine 9 on histone H3 (H3K9ac), acetylated lysine 18 on histone H3 (H3K18ac), acetylated lysine 5 on histone H4 (H4K5ac), and acetylated lysine 12 on histone H4 (H4K12ac). DNA was stained with propidium iodide. Three independent experiments were performed and similar results were obtained. Bar = 20 μm.
Expression of histone-modifying enzymes

As described above, various histone modifications are altered dramatically during oocyte growth (Figs 2 and 4). Given that enzymes that catalyze methylation or acetylation of histones have been identified, we examined the expression of genes that encode these histone-modifying enzymes during oocyte growth to elucidate which enzymes are involved in altering histone modifications during this period.

Histone acetylation is catalyzed by a class of enzymes known as histone acetyltransferases (HATs). Since global histone acetylation of H3K9, K18, and H4K5, K12 prominently increases during oocyte growth (Fig. 2), we examined the expression of SRC-1, p300, PCAF, CBP, HAT1, and TIP60, which have been shown to acetylate these lysine residues in somatic cells (Lachner et al. 2003, Peterson & Laniel 2004, Kikuchi et al. 2005) during oocyte growth (Fig. 7 and Supplemental Fig. S4). The src-1, p300, and pcaf genes exhibited similar patterns of expression: relatively low expression in 5-day-old oocytes, slightly increased expression in 10-day-old oocytes, and markedly increased expression in 15-day-old oocytes. These changes in the expression levels corresponded to those of acetylated H3K9, H3K18, and H4K12, but not that of H4K5 (Fig. 2). Since SRC-1, p300, and PCAF catalyze the acetylation of H3K9 (SRC-1; Peterson & Laniel 2004), H3K9, H3K18, and H4K12 (p300; Lachner et al. 2003), and H3K9 (PCAF; Kikuchi et al. 2005) respectively, these enzymes may be involved in the elevated acetylation of these lysine residues during oocyte growth. Although it is known that p300 also catalyzes the acetylation of H4K5, and its expression level increased markedly in 15-day-old oocytes, the acetylation level of H4K5 did not increase prominently at that time point (Fig. 2). Therefore, the role of p300 in oocytes may be different from that in somatic cells. On the other hand, no obvious change in expression level was detected for cbp, hat1 or tip60 during oocyte growth (Fig. 7).

Histone lysine methylation is catalyzed by histone methyltransferases with the SET domain. SET7, SMYD3, and MLL are known to increase the methylation of H3K4 (Wang et al. 2001, Hamamoto et al. 2004, Smith et al. 2004). The expression level of set7, which is known to be associated with di-methylation but not tri-methylation of H3K4, increased continuously during oocyte growth (Fig. 8 and Supplemental Fig. 5). Since this pattern of change is similar to that of the H3K4me2 (Fig. 4), SET7 may be involved in this type of methylation throughout oocyte growth. The expression levels of smyd3 and mll, which are associated with tri-methylation of H3K4, were relatively low in the 5- and 10-day-old oocytes, markedly increased in the 15-day-old oocytes, and decreased in the GV-stage oocytes (Fig. 8 and Supplemental Fig. 5). Since the level of H3K4me3 showed only a slight increase on day 15 (Fig. 4), it is unclear whether these enzymes are associated with tri-methylation of H3K4 during oocyte growth.

Suppressor of variegation 3–9 homolog 1 (SUV39H), ESET, G9a, and its homolog GLP are known to be H3K9 methyltransferases. The expression levels of all these enzymes increased between days 5 and 10 and then increased markedly on day 15 (Fig. 8 and Supplemental Fig. 5). Since G9a and GLP are known to catalyze di-methylation of H3K9, the elevated levels of these enzymes may be involved in increasing H3K9me2 during oocyte growth (Fig. 4). SUV39H and ESET have been reported to catalyze tri-methylation of H3K9. However, only a marginal increase in H3K9me3 was observed on day 15 (Fig. 4). Therefore, it is unclear whether SUV39H or ESET is associated with tri-methylation of H3K9 during oocyte growth.

Discussion

In the present study, we examined various histone modifications in growing oocytes, in which the global gene expression level decreases with growth. Recent studies have shown that various histone modifications play important roles in the regulation of gene expression...
in mitotic cells. Some modifications, e.g. acetylation of most of the lysine residues in histones H3 and H4 and methylation of H3K4, up-regulate transcription, while other modifications, e.g. methylation of H3K9, down-regulate transcription (Jenuwein & Allis 2001, Kurdistani et al. 2004, Bernstein et al. 2005). However, our results show that the levels of all these histone modifications increase during oocyte growth. Although transcriptional activity decreased with oocyte growth (Worrad et al. 1994), the levels of acetylated histones and methylated H3K4, which are associated with active gene expression, increased (Figs 1–4). Therefore, it is possible that these increases may be involved in genome-wide alteration of chromatin configuration, which is not associated with transcription. Chromatin configuration is altered globally during oocyte growth; the NSN-type configuration is changed to the SN-type configuration (Debey et al. 1993, Zuccotti et al. 1995). It has been reported that this change in chromatin configuration is not involved in decreasing transcriptional activity (De La Fuente et al. 2004).

Comparisons of the epigenetic modifications in SN- and NSN-type oocytes revealed that all of the modifications examined were non-equivalent between these two types of oocytes (Fig. 6). Although it has been reported that DNA methylation and histone modifications contribute to a mechanism that can alter chromatin structure (Jenuwein & Allis 2001), it is not...
Epigenetic modifications in oocytes

Figure 4. Quantification of histone methylation in growing oocytes. Growing oocytes, collected from 5-, 10-, and 15-day-old mice, and GV-stage oocytes were immunostained with antibodies against di-methylated lysine 4 on histone H3 (H3K4me2), tri-methylated lysine 4 on histone H3 (H3K4me3), di-methylated lysine 9 on histone H3 (H3K9me2), and tri-methylated lysine 9 on histone H3 (H3K9me3), followed by treatment with FITC-conjugated secondary antibodies. The fluorescence levels of the oocytes were measured as described in the Materials and Methods section. The average value for GV-stage oocytes is set at 100%, and the values for the growing oocytes are expressed relative to this value. Bars represent S.E.M. In each group, 20–22 oocytes were analyzed.

clear whether changes in histone modifications cause the alteration from the NSN-type to the SN-type chromatin configuration in oocytes. Nevertheless, our results suggest that increases in epigenetic modifications are associated with the acquisition of meiotic and developmental competences (Eppig & Schroeder 1989, Schultz 2002). Small growing oocytes do not have the competence to complete meiotic maturation. During growth, they acquire this competence, which is first observed by day 15 post partum. However, these oocytes still do not have the ability to accomplish preimplantation development. This competence is acquired by day 22 post partum. In our study, most of the epigenetic modifications showed marked increases in 15-day-old oocytes and further increases in GV-stage oocytes. Furthermore, GV-stage oocytes with the NSN-type chromatin configuration, which are developmentally incompetent (Liu & Aoki 2002), showed lower levels of epigenetic modifications when compared with developmentally competent SN-type oocytes (Fig. 6). Therefore, increases in epigenetic modifications, which are associated with changes in chromatin configuration, may play an important role in the acquisition of meiotic and developmental competences during oocyte growth.

Although both H3K9me2 and H3K9me3 increased during oocyte growth, the patterns of these changes and their intra-nuclear localizations differed (Figs 3 and 4). Studies have suggested that di- and tri-methylation exert a similar function, which is to suppress gene expression (Arney & Fisher 2004). However, very recent reports have suggested that the functions of di- and tri-methylation are different. H3K9me2 localizes to the silent domains within euchromatin and facultative heterochromatin, such as the mammalian inactive X-chromosome, and functionally represses transcription (Rougeulle et al. 2004, Tachibana et al. 2005). On the other hand, H3K9me3 is associated with the formation of highly condensed regions of the genome, which are termed constitutive heterochromatin (Arney & Fisher 2004). These findings are consistent with our results showing that in growing oocytes, H3K9me2 was evenly localized throughout the nucleus, in which euchromatin may be spread uniformly, and that H3K9me3 co-localized with the condensed DNA domains, which may represent heterochromatin regions (Fig. 3). Therefore, the increases in di- and tri-methylation of H3K9 may be involved in the suppression of gene expression in the euchromatin and the formation of heterochromatin in growing oocytes.
the oocyte genome, which would lead to the silencing of the entire genome during oocyte growth.

Our results show that the level of DNA methylation increases markedly in 10-day-old growing oocytes (Fig. 5). Since it has been reported that DNA methylation of imprinting genes is established between days 10 and 15 in oocytes (Lucifero et al. 2004), the increase in genome-wide DNA methylation seems to reflect this phenomenon during this period. However, we also found that genome-wide DNA methylation increased after day 15 (Fig. 5). It has been suggested that de novo DNA methylation is catalyzed by Dnmt3b and Dnmt3L (Lyko et al. 1999, Suetake et al. 2004). The expression of dnmt3b and dnmt3L mRNAs was observed in GV-stage oocytes as well as in 15-day-old oocytes (La Salle et al. 2004, Lucifero et al. 2004). Therefore, it is possible that these enzymes catalyze DNA methylation in regions of the genome other than imprinted genes until the GV stage. Indeed, DNA methylation was spread over the entire region in which DNA was detected in the nuclei of GV-stage oocytes. Although the role of this type of global DNA methylation is not clear, a recent study has shown that DNA methylation inhibits the mobilization of transposons (Kato et al. 2003). Although this system of genome transformation is important for the acquisition of genome diversity, germ cells need to maintain stability of their genomes, so as to pass them onto the next generation (Lisch 2002, Kazazian 2004). Therefore, genome-wide DNA methylation may repress, transiently, transposon mobilization during oocyte growth.

We examined the expression of various enzymes that catalyze histone modifications and found candidate enzymes possibly associated with an increased abundance of histone modifications during oocyte growth (Figs 7 and 8). Although the RT-PCR method is not the best method for detecting slight differences in the amounts of transcripts, the changes in the amounts of these enzymes were significantly higher for the SN-type than for the NSN-type oocytes, for all of the epigenetic modifications examined (P < 0.05; Student’s t-test). In each group, 17–30 oocytes, which had been obtained from 2–3 independent experiments, were analyzed. Bar = 20 μm.
**Epigenetic modifications in oocytes**

In the present study, we examined the changes in various epigenetic modifications and in the expression of the enzymes that catalyze histone modifications during oocyte growth. The results of these analyses provide valuable clues as to the roles of epigenetic modifications in mechanisms that regulate cellular functions during oocyte growth.

**Acknowledgements**

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

**References**


