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Meiotic Competence and Acetylation Pattern of UV Light Classified Mouse Antral Oocytes After Meiotic Arrest With Isobutylmethylxanthine

SAFIRIYU IDOWU OLA,[1,2] QIANG WANG,[1] JUN-SHU AI,[1] SHEN YIN,[1] CHENG-GUANG LIANG,[1] DA-YUAN CHEN,[1] AND QING-YUAN SUN[1]*

[1]*State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China*
[2]*Department of Animal Science, Obafemi Awolowo University, Ile-Ife, Nigeria*

ABSTRACT Chromatin transformation from a diffused or NSN configuration to a compacted or SN shape that forms a ring around the nucleolus is regarded as one of the modifications necessary for successful embryonic development. But the process of the transformation is poorly understood. In this study we cultured mouse antral oocytes under meiotic arrest with IBMX for periods between 3 and 24 hr. We observed the chromatin status of the oocytes before and after culture under UV illumination. We reported here that the NSN configured oocytes transformed temporally through an intermediate form into the SN configuration while under meiotic arrest in vitro. Meiotic rate was improved in the NSN oocytes after the meiotic arrest but decreased in the SN oocytes. We also reported that chromatin of both the NSN and SN oocytes was acetylated and the two groups underwent the same pattern of H4/K5 deacetylation during meiotic maturation. We hypothesized that the transformation of mouse oocyte from the NSN to SN type may be time rather than oocyte size specific and the abrupt deacetylation of NSN oocyte during spontaneous maturation may explain its poor meiotic and developmental competence. *Mol. Reprod. Dev. 74: 591–599, 2007.* © 2006 Wiley-Liss, Inc.

Key Words: meiotic arrest; histone (de)acetylation; chromatin configuration; mouse oocyte

INTRODUCTION

The chromatin of the mouse antral oocytes is organized into two conformations: (a) surrounded nucleolus (SN) in which chromatin forms a ring around the nucleolus and also a threadlike pattern in the rest nuclear space; and (b) nonsurrounded nucleolus (NSN) in which the ring is absent but chromatin forms a homogenous diffused nuclear matrix (Mattson and Albertini, 1990; Wickramasinghe et al., 1991; Zuccotti et al., 1995). The intermediate type termed pSN or pNSN (p for partially) is also found though in a smaller proportion (Zuccotti et al., 1995). Ample experimental results suggest that the NSN type oocyte is the immature one that eventually metamorphose into the SN type oocyte during follicullogenesis to guarantee full-term embryonic development (Zuccotti et al., 2005; De La Fuente, 2006). However, when antral oocytes are liberated from their follicular environment, both the SN and NSN types have the capacity to undergo spontaneous maturation and form the MII oocytes, although at a slower and lower rate in the NSN type (Debey et al., 1993; Zuccotti et al., 1998, 2002). The exact mechanism that controls the metamorphosis of the NSN to SN oocyte and whether NSN oocytes are per chance ovulated, especially after superovulation treatment, are not known.

Because antral oocytes punctured out of their follicular environment are at various stages of nuclear and cytoplasmic maturation, this asynchronous situation is partly blamed for the sub-optimal embryonic development in vitro compared to in vivo (Eppig et al., 1994). It is hypothesized that a period of meiotic arrest in vitro before meiotic resumption could synchronize nuclear and cytoplasmic maturation of the oocytes; thereby enhance their meiotic and developmental competence in vitro. Guixue et al. (2001), Nogueira et al. (2003), and Somfai et al. (2003) did show this hypothesis to be true in cattle, mouse, and pig, respectively. Nogueira et al. (2003) further reported that after 24 hr inhibition of the spontaneous meiotic resumption with Org 9935, a PDE3 inhibitor, the proportion of the SN oocytes was higher (82%) than in the untreated oocytes (60%). They assumed that a significant number of the NSN oocytes changed to the SN type during the in vitro meiotic arrest, even though they did not determine the proportions of the NSN and SN oocytes before and after Org 9935 treatment in the same group of oocytes. In our experiment reported here we directly observed the NSN configured oocytes temporally changed to the SN

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*Correspondence to: Qing-Yuan Sun, PhD, State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, #25 Beisihuanxi Road, Haidian, Beijing 100080, China.
E-mail: sunqy@ioz.ac.cn

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configuration during meiotic arrest with isobutylmethylxanthine (IBMX). We thus provide direct evidence to associate the improved meiotic competence of the oocytes after in vitro meiotic arrest period with the metamorphosis from NSN to SN status.

Histone acetylation as one of the major epigenetic mechanism has received in-depth investigation with mouse oocyte and pre-implantation embryo. Thus, it is well established that mouse GV oocytes are fully acetylated at all the lysine residues on H3 and H4 but undergo deacetylation after GVBD and become acetylated again after the formation of one-cell embryo (Kim et al., 2003; De La Fuente, 2006). An acetylated state is associated with a state of transcriptional activity while the deacetylated state corresponds to transcriptional repression (Fuks, 2005). Also NSN oocytes have been shown to be transcriptionally active while the SN oocytes are in a relatively transcriptional quiescent state (Debey et al., 1993; Bouniol-Baly et al., 1999; Christians et al., 1999; Liu and Aoki, 2002). Therefore it will be expected that the GV NSN oocytes would be in an acetylated state and SN oocytes deacetylated state. On the contrary both types of GV oocytes are fully acetylated despite their different transcriptional status (De La Fuente et al., 2004b). Evidence of other important physiological roles of an acetylated status of the GV oocytes have been advanced to explain this paradox ((Ekwall et al., 1997; Aoki and Schultz, 1999; Imai et al., 2000; Suka et al., 2002; Zhang et al., 2002). However, it is not elucidated whether or not both types of oocytes also undergo the same pattern of deacetylation during the maturation period leading to egg formation. We report here similar H4/K5 (de)acetylation pattern in the SN and NSN type oocytes during meiosis though the UV illumination method of classifying oocytes disrupted normal chromosome alignment and segregation.

MATERIALS AND METHODS

Animals and Reagents

Animal care and handling were conducted in accordance with policies on the care and use of animals promulgated by the ethical committee of Institute of Zoology, Chinese Academy of Sciences. The mice with color gene type of aabbcc were from an inbred strain of Kunming white mice, a native breed widely used in biological research in China. All chemicals used in this study were purchased from Sigma Chemical Company (St. Louis, MO) except for those specifically mentioned.

Media and Antibodies

M2 medium with or without 0.2 μM IBMX and 0.05 μg/ml Hoechst 33258 stain supplements was used to receive GV oocytes from ovaries. M16 medium was used to culture GV oocytes to MII stage. Rabbit polyclonal anti-acetyl lysine 5 in histone 4 (AcH4/K5) and AcH4/K12 antibodies were obtained from Upstate Biotechnology (Lake Placid, NY); goat polyclonal AcH4/K16 from Santa Cruz Biotechnology (Santa Cruz, CA), and rabbit polyclonal AcH3/K9 from Cell Signalling Technology (Beverly, MA). Fluorescein isothiocyanate (FITC) conjugated goat anti-rabbit and mouse anti-goat secondary antibodies were obtained from Santa Cruz Biotechnology.

Oocyte Isolation, Classification, and Culture

Ovaries were aseptically collected from 4–8 weeks old mice into pre-warmed M2 medium supplemented with 0.2 μM IBMX and minced with a clean sharpener for about 10 min to liberate the antral oocytes. Cumulus-free oocytes with diameter >70 μm were selected and washed in M2 with or without Hoechst 33258 stain (0.05 μg/ml) depending on whether or not classification based on chromatin organization into SN or NSN oocytes was required. The classification was done according to the procedure described by Zuccotti et al. (2002). Briefly, oocytes were cultured in groups in 50 μl of M2 supplemented with 0.2 μM IBMX and 0.05 μg/ml Hoechst 33258 at 37°C for 15 min. Thereafter oocytes were transferred individually into 5 μl droplet of M2 containing 0.2 μM IBMX under mineral oil in a 35-mm plastic dish (Hunngyan, Taizhan, China). Thirty micro-drops were made in one dish. Then oocytes were examined under UV illumination of an inverted fluorescence microscope (Nikon eclipse TE 300) at 400× magnification. Each oocyte was exposed to the UV illumination for not more than 5 sec during which we specifically checked for the presence of a ring of Hoechst-positive chromatin around the nucleolus (depicting SN oocyte) or its absence (depicting NSN oocyte).

Depending on each experimental objective both classified and unclassified groups of oocyte were cultured as highlighted below.

Experiment 1: Temporal Changes in Mouse Oocyte Chromatin Configuration In Vitro

To determine whether changes would occur in the chromatin configuration of mouse antral oocytes after liberation from the follicle in vitro, denuded oocytes after classification under brief UV light exposure were further incubated in the same medium at 37°C and 5% CO_2 for 3, 6, 9, 12, or 24 hr. At the end of these periods the chromatin architecture of the oocytes was re-checked under a longer time UV illumination. In the 24 hr group the diameter of the oocytes at the start and end of incubation was also recoded.

Experiment 2: Meiotic Competence of SN and NSN Mouse Antral Oocytes After Meiotic Arrest

To verify if a temporal change in the chromatin organization in vitro as observed in experiment 1 would translate into better meiotic rate, classified SN and NSN oocytes were matured separately in M16 medium for 15 hr with or without prior incubation with IBMX. Because we suspect Hoechst stain might have some harmful effects on oocyte performance, we set up control treatments with unclassified oocytes to separate the effects of Hoechst staining and UV light exposure on meiotic maturation of the oocytes. A schematic representation of experiment 2 is shown in Figure 1.

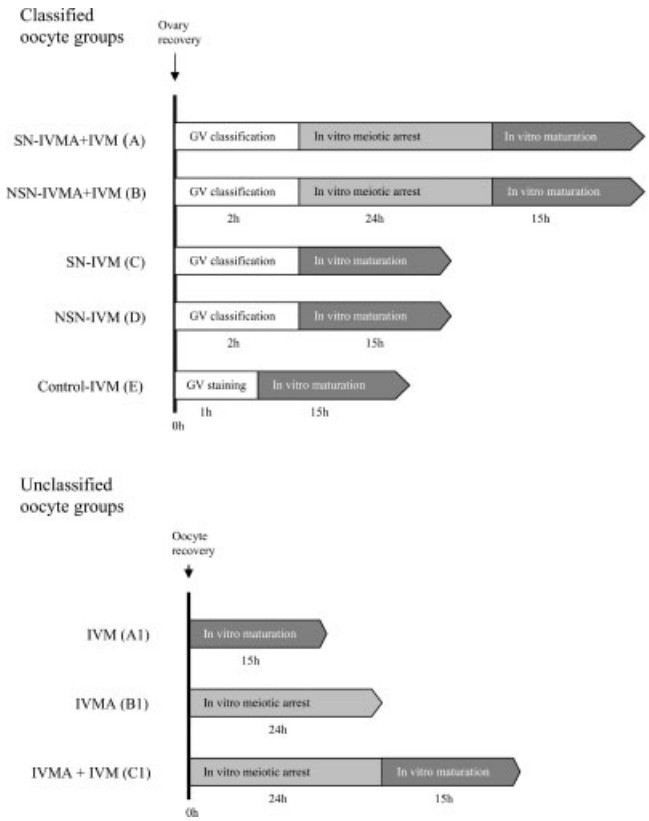

Fig. 1. Schematic illustration of experiment 2. SN, surrounded nucleolus; NSN, nonsurrounded nucleolus; IVM, in vitro maturation; IVMA, in vitro meiotic arrest.

Experiment 3: Histone Acetylation Pattern During Meiotic Maturation of Classified Oocytes

To determine whether both SN and NSN oocytes follow the same pattern of deacetylation during meiotic maturation, antral oocytes were classified as described above and then matured separately in M16 at 37°C for 15 hr. Samples of the oocytes were taken at 0, 4, 10, and 15 hr of culture and fixed in 4% paraformaldehyde in PBS (pH 7.4) for 30 min, permeabilized with 0.5% Triton X-100 for 20 min and then blocked in 1% BSA-supplemented PBS (blocking solution) for 1 hr at room temperature. Oocyte samples obtained at 0 hr of culture (GV oocytes) were incubated with either anti-AcH4/K5 (1:200) or AcH3/K9 (1:200) or AcH4/K12 (1:200) or AcH4/K16 (1:50) antibodies overnight at 4°C. Samples retrieved at the other time periods were probed only with AcH4/K5 antibody. Next, fixed oocyte samples were counter labeled with FITC conjugated secondary antibody (1:100) for 1 hr in a dark box at room temperature. Finally oocytes were washed, stained with 7.5 μg/ml Hoechst 33258 (GV oocytes) or 10 μg/ml propidium iodide (GVBD to MII oocytes) for 15 min and mounted as elaborated by Zhang et al. (2005).

Immunofluorescence Analysis

Cells were examined with Confocal Laser-Scanning Microscope (Zeiss LSM 510 META, Germany). Images were acquired by sequential excitation at 488 nm and 543 nm laser lines. Instrument settings were kept constant in each replicate. The images were then adjusted only for brightness and contrast using linear scaling of the minimum and maximum intensities.

Statistical Analysis

Each experiment was repeated three times. Data from experiment 1 and 2 are presented as mean percentages ± SD. Statistical differences between treatment groups was checked with one-way ANOVA and Duncan tests of the SPSS 13 software. Difference at $P < 0.05$ was considered significant.

RESULTS

Experiment 1: Temporal Transformation of Mouse Oocyte Chromatin Organization During In Vitro Meiotic Arrest

Mouse oocytes classified into SN (n = 308) or NSN (n = 336) were incubated at 37°C, 5% CO_2 in M16 medium containing 0.2 μM IBMX to arrest meiotic maturation for 3-, 6-, 9-, 12-, or 24-hr period. The chromatin architecture was checked before and after the incubation period. SN configured oocytes maintained their configuration irrespective of the length of meiotic arrest whereas in the NSN oocytes different types of configuration have evolved at the end of the incubation. Figure 2 shows the proportion of NSN, intermediate,

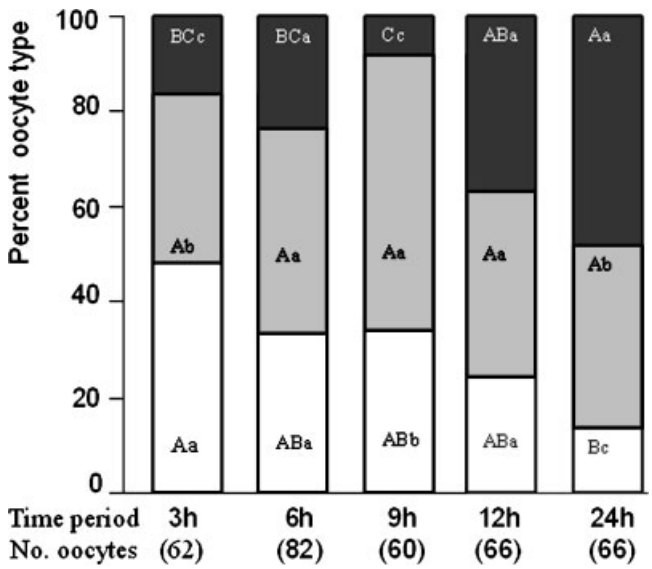

Fig. 2. NSN to SN conversion rate during in vitro meiotic arrest. □ NSN; ▒ Intermediate; ■ SN. Each culture period was replicated 3 times with a minimum of 20 oocytes each time. The stacked bars represent the mean% of the replicates. Figures in bracket represent the total number of NSN oocytes cultured at each time period. SD ranged from ± 3.33% (for SN configuration at 3-hr culture) to ± 22.23% (for intermediate configuration at 8-hr culture). ABC and abc indicates significant difference ($P < 0.05$) between columns of different stacked bars and between columns of each stacked bar, respectively. At each time period all the starting oocytes were of NSN configuration with diameter >70 μm. The chromatin configuration of all the oocytes was then rechecked at the end of the period of meiotic arrest.

and SN oocytes at the end of each incubation period from an initial 100% NSN population. After 3 hr of meiotic arrest in vitro, $35.58 \pm 4.38\%$ and $16.21 \pm 3.75\%$ of the initial NSN oocytes population presented the intermediate and SN configurations, respectively, while the remaining $48.21 \pm 8.13\%$ retained the NSN configuration. There was an apparent decline in the proportion of the NSN configuration (from $48.21 \pm 8.13\%$ to $13.51 \pm 5.97\%$) with a corresponding increase in that of the SN (from $16.21 \pm 3.75\%$ to $47.93 \pm 10.02\%$) while that of the intermediate configuration remained around $43.25 \pm 10.08\%$ as the culture period increased from 3 to 24 hr. At these two extreme periods both the intra- and inter-group difference in the proportions of the SN and NSN oocytes were significant ($P < 0.05$). Thus it is evident that the NSN oocytes transformed into the SN type through the intermediate type (Fig. 3) over time in vitro. The conversion of NSN oocytes to full SN oocytes during in vitro meiotic arrest also appeared to be accompanied by a significant increase in size (Table 1). The diameter (including the Zona Pellucida) of the NSN oocytes increased by an average of 3.58 μm in the process of becoming SN oocytes during the 24 hr in vitro meiotic arrest. On the contrary some of the initial SN oocytes which retained the configuration had slightly reduced size while many had collapsed cytoplasm and few became cloudy (degenerated). Such occurrences were not noticed in the NSN oocytes. However, SN oocytes were significantly ($P < 0.001$) larger than the NSN oocytes both before and after meiotic arrest.

Experiment 2: Meiotic Competence of SN and NSN Mouse Oocytes After Meiotic Arrest

SN and NSN oocytes were matured separately in M16 medium for 15 hr without prior or after 24 hr meiotic arrest with IBMX. The control group for this trial included oocytes that were collected in and thereby stained with Hoechst containing medium (0.05 μg/ml) but were not subjected to UV illumination. This was aimed at balancing the potential effect that Hoechst staining might have on the maturation process. All the SN oocytes ($n = 111$) that were kept meiotic arrest before maturation culture (group C) underwent GVBD whereas $19.37 \pm 9.65\%$ and $25.03 \pm 4.55\%$ in the SN (B) and control (A) groups, respectively, cultured soon after collection, still remained at GV stage (Table 2). Similarly $34.73 \pm 0.64\%$ of NSN oocytes arrested before maturation culture (E) remained at GV stage compared to $69.10 \pm 14.50\%$ in the NSN group cultured without arrest (D). Meiotic arrest also led to higher percentage of NSN oocytes reaching MII stage ($32.17 \pm 2.71\%$ in group E against $14.57 \pm 5.45\%$ in group D), although both figures were still lower to those of SN and control groups. The reverse was the case in the SN oocytes as more oocytes reached MII stage when cultured soon after collection ($58.07 \pm 6.45\%$ in group B) compared to when arrested for 24 hr before allowed maturation-culture ($43.23 \pm 5.45\%$ in group C). A significantly ($P < 0.05$) higher percentage ($16.20 \pm 5.40\%$) of SN oocytes arrested before maturation also became degenerated during the process compared culture to less than 6% in the other groups.

In an attempt to clarify the effect that Hoechst staining and UV illumination for categorizing oocytes into SN or NSN might have on the subsequent maturation process, we performed a second trial with unclassified oocytes cultured soon after isolation or arrested for 24 hr before maturation. As shown in Table 3 unclassified oocyte cultured soon after isolation (group A1) had lower MII rate ($50.95 \pm 5.95\%$) and higher GV arrested stage ($29.90 \pm 0.15\%$) compared to the arrested before maturation oocytes, group C1 ($61.60 \pm 5.60\%$ and $14.50 \pm 4.20\%$, respectively). IBMX at the 0.2 μM used in this study arrested spontaneous maturation in 99% of the denuded oocyte (group B1) but as many as $10.70 \pm 2.25\%$ became degenerated by the end of the arrest. A numerical comparison between the Hoechst stained control (group A; Table 2) of trial 1 and unclassified control (group A1; Table 3) of trial 2 showed they had similar figures for each stage of the meiotic maturation process, thus removing doubts of potential harm to maturation progression as a result of Hoechst staining.

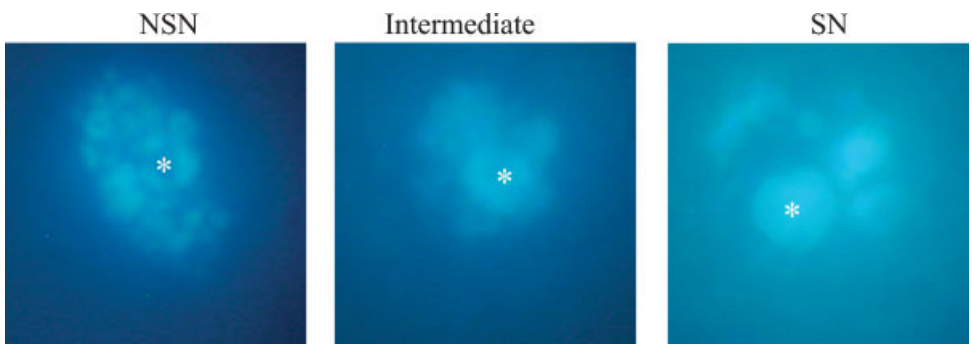

Fig. 3. Transition of mouse oocyte from NSN chromatin configuration through the intermediate type to the SN configuration. Asterisks denote the position of nucleolus. Note the diffuse chromatin in the NSN and intermediate and the chromatin ring around nucleolus in the intermediate and SN. The images were shot after 24 hr of meiotic arrest with Nikon Coolpix 950 digital camera attached to Nikon TE 300 fluorescence inverted microscope. Magnification 400×.

TABLE 1. Changes in Oocyte Size (μm) During 24-hr In Vitro Meiotic Arrest

Chromatin reorganisation	Size before arrest	Size after arrest	*P*-value
NSN to NSN	73.0 ± 2.24 (9)	75.0 ± 4.66 (9)	0.357
NSN to intermediate	70.4 ± 0.89 (24)	72.80 ± 2.03 (24)	0.128
NSN to SN	72.0 ± 1.75a (33)a	75.58 ± 3.31b (33)b	0.001
SN to SN	80.64 ± 2.42 (22)c	80.67 ± 1.94 (18)c	0.687

Values are given as mean ± SD. Figures in bracket represent number of oocytes. Only the oocytes that transformed from NSN to full SN increased in size significantly (a, b; $P < 0.001$). SN oocytes were significantly (c; $P < 0.01$) bigger than NSN at start and end of culture.

Experiment 3: Histone Acetylation Pattern During Meiotic Maturation of Classified Oocytes

To confirm previous reports on histone acetylation status of GV oocytes we first probed mouse antral oocytes of diameter >70 μm with anti-Acetyl H3 on Lysine 9 and H4 on Lysine 5, 12, and 16 antibodies. The confocal laser scanning of the immunofluorescence images is reported in Figure 4. As shown, both the NSN- and SN-configured oocytes were acetylated at all the lysine residues probed. In the figure the Hoechst-stained DNA was not so clear (especially for the NSN oocytes, Figure 4; B2, D2, F2, and H2) because our confocal laser machine was not equipped with a filter for UV light excitation. However, the corresponding antibody fluorescence images depicted closely the chromatin configuration seen under the microscope. In the next trial antral oocytes were first classified into NSN or SN and then cultured separately in maturation medium for 15 hr. At time point 0, 4, 10, and 15 hr corresponding to GV, GVBD, AI, and MII, respectively, samples were fixed and probed with anti-Ac H4/K5 antibody. As shown in Figure 5 both SN and NSN oocytes were deacetylated once GVBD occurred. Another important observation was the lack of chromosome alignment and defective chromosome segregation at periods believed to be MI (Fig. 5; E2,F2) and AI (Fig. 5; G2,H2), respectively. Majority of the oocytes that reached the MII stage were also defective with abnormalities such as large polar body and nonaligned MII chromosomes.

DISCUSSION

Meiotic Competence of Mouse Oocyte After In Vitro Meiotic Arrest

Ever since Pincus and Enzmann (1935) first discovered the capability of mammalian oocyte (working with rabbit) to resume meiotic maturation in vitro once liberated from its granulosa cells surroundings, considerable knowledge has been acquired on this process but our understanding of it is far from being complete. One of the pursue still awaiting explanation is how the oocyte matures from the so-named nonsurrounded nucleolus (NSN) type to become the surrounded nucleolus (SN) type. The later is known to be of higher meiotic and developmental competence (Debey et al., 1993; Zuccotti et al., 1998, 2002). Because other characteristics such as size (Wickramasinghe et al., 1991; Debey et al., 1993; Zuccotti et al., 1995), state of transcription (Debey et al., 1993; Bouniol-Baly et al., 1999; De La Fuente and Eppig, 2001), and level of stored mRNA (Christians et al., 1999; Gentile et al., 2004) all indicates higher maturation status of SN over NSN oocytes, it is agreed that chromatin reorganization from NSN to SN configuration is one of the epigenetic changes required for successful embryonic development (De La Fuente, 2006). But exactly what initiates and propels this transformation and how long it takes to complete this process are not precisely known. SN conformation could be seen as early as in preantral follicle with oocyte diameter of about 50 μm which is just about 63% of the

TABLE 2. Meiotic Maturation of UV Light Classified Mouse Oocyte With or Without Prior in vitro Meiotic Arrest

		SN oocytes		NSN oocytes	
Stage	Control IVM (A)	IVM (B)	IVMA + IVM (C)	IVM (D)	IVMA + IVM (E)
GV	25.03 ± 4.55b,c (33)	19.37 ± 9.65b (18)	0.00 ± 0.00a (0)	69.10 ± 14.50d (114)	34.73 ± 0.64c (49)
GVBD	20.47 ± 6.85a (27)	19.40 ± 12.90a (18)	40.53 ± 13.55b (45)	16.37 ± 7.25a (27)	27.23 ± 4.52a,b (39)
MII	52.30 ± 6.80c,d (69)	58.07 ± 6.45d (54)	43.23 ± 5.45c (48)	14.57 ± 5.45a (24)	32.17 ± 2.71b (45)
DEG	2.27 ± 2.25a (3)	3.23 ± 3.25a (3)	16.20 ± 5.40b (18)	0.00 ± 0.00a (0)	5.90 ± 1.90a (8)
Total No of oocyte	132	93	111	165	141

IVM, in vitro maturation; IVMA, in vitro meiotic arrest; GV, germinal vesicle; GVBD, germinal vesicle breakdown; MII, metaphase II; and DEG, degenerated oocytes. Oocytes were either cultured soon after isolation (A, B, D) or arrested with IBMX for 24 hr before maturation culture (C and E). Values represent mean % ± SD of three replicates. Figures in bracket represent number of oocytes. a, b, c, d indicate significant difference between values on the same row at $P < 0.05$.

TABLE 3. Meiotic Maturation of Unclassified Mouse Oocyte With or Without Prior In Vitro Meiotic Arrest

Stage	IVM (A1)	IVMA (B1)	IVMA + IVM (C1)
GV	29.90 ± 0.15b (54)	88.30 ± 3.30a (268)	14.50 ± 4.20c (32)
GVBD	16.10 ± 0.56a (30)	0.00 ± 0.00b (0)	14.40 ± 5.10a (30)
MII	50.95 ± 5.95b (89)	1.00 ± 1.00c (3)	61.60 ± 5.60a (132)
DEG	3.05 ± 1.25b (6)	10.70 ± 2.25a (33)	9.45 ± 4.55a (21)
Total No of oocytes	179	304	215

IVM, in vitro maturation; IVMA, in vitro meiotic arrest; GV, germinal vesicle; GVBD, germinal vesicle breakdown; MII, metaphase II; and DEG, degenerated oocytes. Values represent mean % ± SD of three replicates. Figures in bracket represent number of oocytes. a, b, c indicates significant difference between values on the same row at $P < 0.05$.

final size (Zuccotti et al., 1995; Bouniol-Baly et al., 1999). Our study here suggested that transformation from NSN to SN may not be size- but rather time-specific once initiated, as 48.21 ± 8.13% and 13.51 ± 11.97% of NSN oocytes remained as NSN after 3 and 24 hr, respectively, of in vitro meiotic arrest. Both groups were started with oocyte population of average diameter >70 μm. We hypothesized that certain physiological changes trigger the transformation which would be completed within a time schedule.

Our study also supported the notion that developmental competence of oocyte would improve after a period of meiotic arrest in vitro (Sirard, 2001; Nogueira et al., 2003; Somfai et al., 2003). We viewed the transformation of the NSN type oocyte through the intermediate type to the SN type (Fig. 3) under meiotic arrest with IBMX as part of the global epigenetic modifications occurring in vitro. Nogueira et al. (2003) showed that mouse oocytes maintained under meiotic arrest with Org 9344, a PDE3 inhibitor, for 24 hr exhibited better

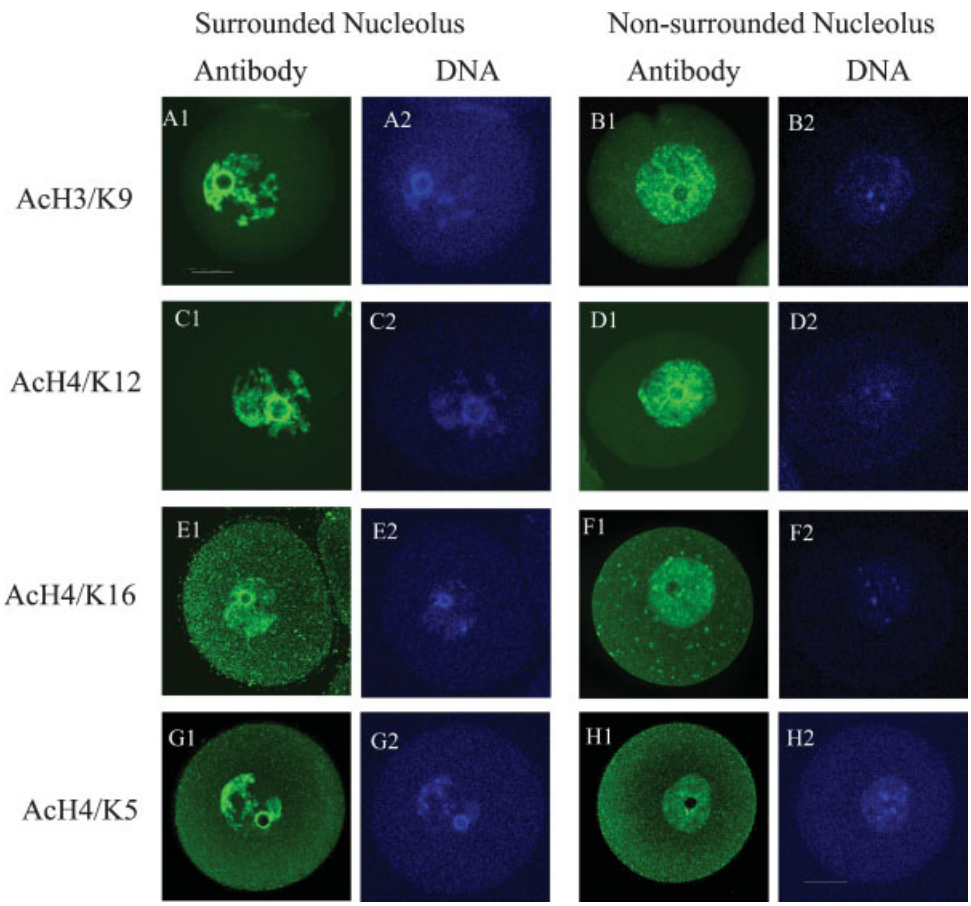

Fig. 4. Histone acetylation status of mouse GV oocyte. Both the SN and NSN GV oocyte types were acetylated. DNA was stained with Hoechst 33258. Bars = 20 μm.

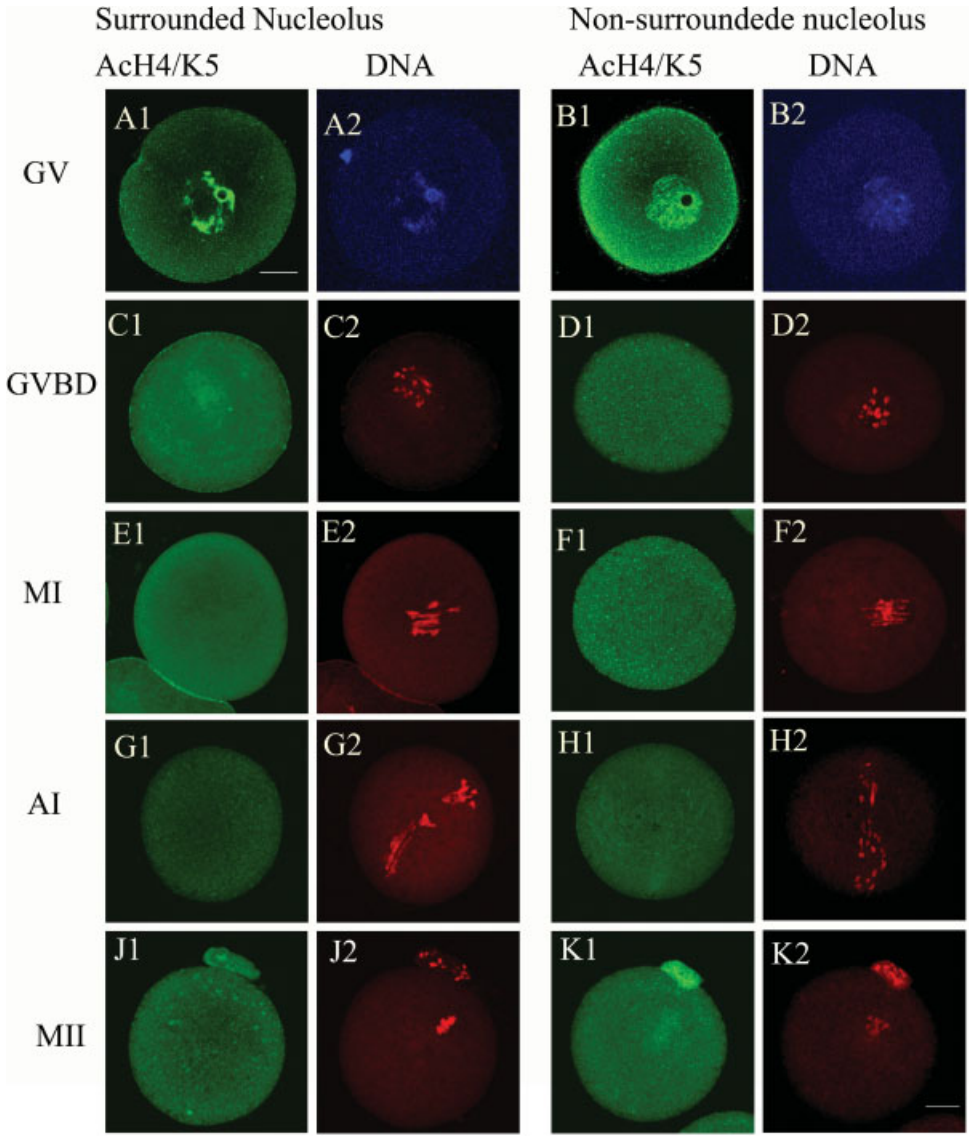

Fig. 5. H4/K5 deacetylation during meiotic maturation of SN and NSN mouse oocytes. GV-germinal vesicle stage; GVBD, germinal vesicle breakdown; MI, metaphase I; AI, anaphase I; and MII, metaphase II stages. Both SN and NSN oocyte types were deacetylated following GVBD until MII formation (C1 to K2). Note the nonalignment of chromosomes at MI stage (E2 and F2) and the improper chromosome segregation at A1 (G2 and H2) and the nonaligned chromosomes at MII in the NSN (K2). Chromosomes at GV and later stages were stained with Hoechst 33258 (blue fluorescence, figures A2 and B2) and Propidium iodide (red fluorescence), respectively. Bars = 20 μm.

meiotic and developmental ability. They also reported a higher proportion of SN oocytes after the meiotic arrest (82%) compared to the untreated population (60%) and provided indirect evidence of the transformation from NSN to SN in vitro. Our results here provided the direct evidence that transformation from NSN to SN did occur during the meiotic arrest in vitro. It would be of interest to know whether the intermediate type oocyte has similar meiotic and developmental capability as the full SN type. It appears in vitro arrest has differential effects on SN and NSN oocytes. In our study, the meiotic competence was apparently improved after the arrest in the NSN oocytes but decreased in the SN oocytes. This observation could be explained with a number of reasons: (1) ageing effect: this could be supported by the significantly higher percentage of degenerated oocytes during in vitro arrest in the SN compared to NSN groups (Table 2). De La Fuente and Eppig (2001) reported that cleavage rate and frequency of blastocyst formation were reduced when pre-ovulatory mouse GV oocytes were maintained in prolonged meiotic arrest for up to 4–6 days. They adduced this effect to the increased lag between the global transcriptional repression and resumption of meiosis; (2) denudation effect: at least in the mouse model, cumulus granulosa cells that remain in contact with the oocyte are believed to perform some critical role in the developmental regulation of global transcriptional silencing in pre-ovulatory oocytes. In the absence of a patent gap junctional communication with somatic granulosa cells, transcriptional activity

remained unabated in denuded oocytes (De La Fuente and Eppig, 2001). Such effect could work against subsequent meiotic maturation and development. Earlier Eppig et al. (1997) and Simon et al. (1997) reported that both the growth and meiotic rate were lowered in denuded oocyte compared to cumulus enclosed oocytes, the latter behaved more like in vivo matured oocytes (De La Fuente and Eppig, 2001). Sun et al. (2001) equally reported that cumulus cells-oocyte communication during maturation enhances cytoplasmic maturation and embryonic development in pig. Thus, oocyte communication with the somatic cell compartment seems essential for both oocyte growth and acquisition of meiotic competence. However, the fact that classification with Hoechst staining is impossible in cumulus enclosed oocyte made denudation in our experiment necessary.

Despite the apparent deleterious effect of 24 hr meiotic arrest on the SN oocytes meiotic competence, the development in SN-arrested group was still better than in the NSN-arrested oocytes. A shorter time of in vitro arrest may benefit the two types. Generally meiotic rate of classified oocytes in our study was around 50%. Our suspicion that the UV illumination exposure (even though as brief as 5 sec) used to classify the oocytes into SN or NSN could have negative impact on meiotic maturation was confirmed in our second experiment on the acetylation status during maturation. As revealed in Figure 5 (E2, F2, G2, H2) both SN and NSN oocytes previously exposed to UV illumination failed to align and segregate chromosomes normally after GVBD. Bradshaw et al. (1995) reported that when bovine oocyte was exposed to UV-C light (254 nm) spindle formation was abrogated, maturation promoting factor level failed to increase and more defective eggs were formed. We obtained similar maturation rates in Hoechst stained (but not UV light exposed) oocytes and the unstained control and thus conclude that the actual negative factor to maturation was the UV light exposure, no matter how short (Velilla et al., 2002). Thus, until a suitable marker or method for classifying oocytes into NSN and SN is developed, the true meiotic competence of both types after classification may not be exactly known.

Histone (De)Acetylation During Meiotic Maturation

Mouse oocytes, matured or immatured, remain acetylated under meiotic arrest both in vivo and in vitro (De La Fuente et al., 2004b). Even though acetylation is associated with transcriptional activity, which is the perfect status of the NSN GV oocyte, acetylation of SN GV oocytes, known to be transcriptionally quite could be attributed to many other roles that have since been associated with an acetylated status. Such functions include heterochromatin formation (Imai et al., 2000; Kimura et al., 2002; Suka et al., 2002), regulation of DNA replication (Aoki and Schultz, 1999; Vogelauer et al., 2002; Zhang et al., 2002) and chromosome segregation (Ekwall et al., 1997). As we have shown in this study histone deacetylation during meiosis occurs in both NSN and SN oocytes. This further reinforces the notion that transcriptional repression and the resumption of meiosis in the GV oocyte may follow different pathways (De La Fuente et al., 2004a). Histone deacetylation is not required for GVBD (De La Fuente et al., 2004a) but once GVBD occurs deacetylation follows (Kim et al., 2003; De La Fuente et al., 2004b; Sarmento et al., 2004). This will be quite normal for a fully grown SN oocyte that is transcriptionally quite but probably results in abrupt seizure of RNA transcription that was hitherto ongoing in the NSN oocyte and which is necessary for embryonic survival (Stebbinsboaz et al., 1996; Hodgman et al., 2001). This could precisely explain why NSN oocytes failed to move beyond the 2-cell stage after fertilization (Zuccotti et al., 1998, 2002).

Decondensation of chromatin in previously condensed SN oocytes does not restore transcriptional activity neither is remodeling chromatin into the SN configuration strictly required for global transcriptional repression in mammalian oocytes (De La Fuente et al., 2004a). This suggests that chromatin structure and transcriptional activity could only be passively related. It will be useful to know if transcriptional repression is automatically induced in NSN oocytes upon resumption of meiosis and what happens if deacetylation is prevented in NSN oocytes. Hyperacetylation during meiosis disrupted the binding of ATRX to centromeric domains and resulted in the formation of abnormal chromosome alignments at the meiotic spindle (De La Fuente et al., 2004b). Deacetylation of H4/K5 is believed to be essential for the recruit of heterochromatin-binding proteins to the centromeric domain (Taddei et al., 2001; De La Fuente et al., 2004b).

CONCLUSIONS

It is apparent that acquisition of the condensed/SN chromatin structure through global epigenetic changes is a signal of meiotic and developmental competence in mouse oocytes. More importantly; however, during the transformation is that synthesis and storage of viable, translationally dormant, maternal products be completed before the onset of global transcriptional silencing. We have shown in this report that meiotic maturation in both SN and NSN configured mouse oocytes is accompanied by H4/K5 deacetylation. If indeed deacetylation correlates positively with transcriptional repression, then the sudden deacetylation of the hitherto transcriptionally active NSN oocyte could explain why it is developmentally incompetent. Meiotic competence of NSN oocytes is improved when maintained in meiotic arrest in vitro for 24 hr which allowed transformation to SN status and probably continuation of other epigenetic modifications. Under the same condition meiotic competence was slightly reduced in the SN oocytes. Thus, a noninjurious method for analyzing chromatin structure at GV stage would enhance the separation of immediately meiotically competent oocytes from those requiring further arrest.

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