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Transcriptional activity associated with meiotic competence in fully grown mouse GV oocytes

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Summary

The involvement of cumulus cells and chromatin organisation in transcriptional activity was investigated. In addition, the relationship between transcriptional activity and meiotic competence in fully grown mouse oocytes was surveyed. Transcriptional activity was detected in fully grown oocytes in which chromatin did not surround the nucleolus in the germinal vesicle (NSN-type oocytes), but not in oocytes in which chromatin surrounded the nucleolus (SN-type oocytes). Cumulus cells seemed to downregulate transcriptional activity in NSN-type oocytes, since transcriptional activity was 3 times greater in the denuded NSN-type oocytes free of cumulus cells (DO oocytes) than in NSN-type oocytes enclosed in cumulus cells (COC oocytes). Higher transcriptional activity corresponded to lower germinal vesicle breakdown (GVB) competence of fully grown oocytes in culture. Although GVB occurred in nearly all (99%) the SN-type oocytes, it occurred in 88% of COC/NSN-type oocytes (cumulus–oocyte complex with SN-type configuration) and in 61% of DO/NSN-type oocytes (denuded oocytes with NSN-type configuration). There was a negative correlation between transcriptional activity and the capacity of a cell to complete the progression to the second metaphase (MII). In GVB oocytes, the percentage of first polar body (PBI) extrusion differed among COC/NSN-type (81%), DO/SN-type (66%), COC/NSN-type (47%) and DO/NSN-type (29%) oocytes. After activation with 10 mM Sr^{2+}, the frequency of parthenogenetic activation was greater in SN-type oocytes (46.9%) than in transcriptionally active NSN-type oocytes (27.5%). These results suggest that transcriptional activity has a detrimental effect on the competence of meiotic maturation and subsequent activation in fully grown GV oocytes. Alternatively, active transcription in the fully grown oocytes suggests that they are still in the process of synthesising substances required for meiotic maturation and are not yet competent for these processes.

Keywords: Chromatin configuration, GV oocyte, Meiotic maturation, Mouse, Transcription

Introduction

Mouse oocytes isolated from follicles are capable of spontaneously completing meiotic maturation *in vitro*. They are heterogeneous in many ways, including the number of attached cumulus cells (Mangia & Epstein, 1975; Swartz & Schuetz, 1976) and nuclear chromatin configuration (Mattson & Albertini, 1990; Debey *et al*., 1993).

Fully grown mouse germinal vesicle (GV) oocytes from large antral follicles can be classified into two groups, according to their chromatin configuration (Debey *et al*., 1993; Zuccotti *et al*., 1995). In the first group, called the surrounded-nucleolus (SN) type, the chromatin is highly condensed and is concentrated in the area around the nucleolus. In the second group, called the non-surrounded-nucleolus (NSN) type, the chromatin is less condensed and does not surround the nucleolus. During oogenesis, oocytes differentiate into either of these two chromatin configurations. Initially, the chromatin configuration in all oocytes is the NSN type (Mattson & Albertini, 1990; Debey *et al*., 1993). As they grow, the configuration in some of these oocytes remains that of NSN type, while it shifts to the SN type

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in others. The percentage of SN oocytes increases with oocyte size and age (Wickramasinghe *et al.*, 1991; Debey *et al.*, 1993; Zuccotti *et al.*, 1995). The SN-type configuration is strictly correlated with the arrest of both pol I- and pol II-dependent transcription at any age. In contrast, both pol I- and pol II-dependent transcriptional activity is detected in NSN-type oocytes (Bouniol-Baly *et al.*, 1999; De La Fuente & Eppig, 2001). The transition from NSN to SN type is a necessary prerequisite for the ovulation of fully grown antral oocytes *in vivo* (Wickramasinghe *et al.*, 1991; Zuccotti *et al.*, 1995). However, both NSN- and SN-type oocytes can mature to metaphase II *in vitro*. Nevertheless, the percentage of oocytes that mature is lower and the progression is slower in NSN-type oocytes; they undergo germinal vesicle breakdown (GVB) 30 min later than SN-type oocytes and extrude the first polar body 30 min later (Debey *et al.*, 1993). The reason for this retarded maturation remains unknown.

Oocytes need to communicate with surrounding somatic cells in order to grow and acquire meiotic competence (Eppig *et al.*, 1997; Simon *et al.*, 1997). The competence to undergo GVB differs between denuded oocytes and the oocytes associated with granulosa cells, which are important regulators of the final events determining the differentiation of growing oocytes (Chesnel *et al.*, 1994; Chesnel & Eppig, 1995).

Although some factors involved in the meiotic competence of fully grown oocytes are known, little is known about the mechanisms involved. The individual effects of cumulus cells and the effects of chromatin organisation on the meiotic competence of mouse oocytes have been studied, but little is known about any synergistic effect of the two stimuli on the frequency of successful maturation. Although the relationship between chromatin configuration and transcriptional activity has been shown (Bouniol-Baly *et al.*, 1999; De La Fuente & Eppig, 2001), it is not known whether cumulus cells attached to fully grown GV oocytes have any effect on transcriptional activity. This study investigated the role of cumulus cells and chromatin organisation in transcriptional activity, and surveyed the relationship between transcriptional activity and meiotic competence in fully grown mouse oocytes.

Materials and methods

Collection and culture of oocytes

Oocytes were collected from 4-week-old ddY mice (SLC, Shizuoka, Japan) by puncturing follicles with a sharp needle in Whitten's medium (Whitten, 1971) supplemented with 20 mM HEPES and 0.2 mM 3-isobutyl-1-methylxanthine (IBMX). Only oocytes with a diameter >70 μm were sorted for further culture. Cumulus cells in cumulus–oocyte complexes were removed by gentle pipetting through a narrow-bore glass pipette. After assessing the chromatin configuration, as described below, oocytes were washed 5 times in IBMX-free Whitten's medium, and further cultured in medium in a humidified atmosphere of 5% CO_2/95% air at 38 °C.

Assessment of chromatin configuration

Oocytes were labelled by adding Hoechst 33342 (Sigma Chemical Co., St Louis, MO) to the medium to a final concentration of 10 ng/ml. After 10 min of culture, the oocytes were distributed individually in small droplets of medium containing the same concentration of Hoechst 33342 in Petriperm dishes under mineral oil. Observations were made using an inverted microscope (Nikon ECLIPSE TE300) equipped with a 100 W mercury lamp (Osram HBO) and a plate heated to 38 °C. Hoechst fluorescence was observed by excitation at 360 nm through a Nikon UV 2A filter block. The intensity of the excitation light (100 W mercury lamp) was attenuated by neutral filters. The duration of oocyte observations did not exceed 5 s. In a preliminary experiment, we found that these conditions did not cause major impairment of oocyte viability. Then, the GV oocytes were classified into four groups with respect to chromatin configuration and the presence of attached cumulus cells: COC/SN (cumulus–oocyte complexes with SN-type chromatin configuration), COC/NSN (cumulus–oocyte complexes with NSN-type chromatin configuration), DO/SN (denuded oocyte with SN-type chromatin configuration) and DO/NSN (denuded oocyte with NSN-type chromatin configuration).

In vitro maturation and parthenogenetic activation of oocytes

Oocytes were matured for 16 h in Whitten's medium. GVB and the extrusion of the first polar body (PBI) were measured 4 and 16 h after culture in IBMX-free medium, respectively. Oocytes at metaphase II were activated with Sr^{2+} (Bos-Mikich *et al.*, 1997). The mature oocytes were incubated for 10 min in Ca^{2+}-free Whitten's medium containing 10 mM Sr^{2+}, and washed and cultured in CZB medium (Chatot *et al.*, 1990). Oocytes with at least one pronucleus 6 h after activation were considered activated.

In vitro transcription assay

The transcription activity of oocytes at GV stage was determined by measuring the incorporation of 5-bromouridine-5-triphosphate (BrUTP; Sigma Chemical Co.) into plasma-membrane-permeabilised

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oocytes as described by Aoki *et al.* (1997) with minor modifications. Briefly, the plasma membranes of oocytes were permeabilised by treatment with 0.05% Triton X-100 for 1–2 min. Permeabilised oocytes were used *in vitro* transcription reactions where UTP replaced BrUTP in the reaction mixture. After a 15 min incubation at 33 °C, oocytes were fixed overnight with 3.7% paraformaldehyde. Permeabilisation of the nuclear membrane was performed on fixed oocytes with 0.2% Triton X-100 for 3 min. Incorporated BrU was detected by immunostaining with an anti-BrdU antibody that also recognises BrU and an anti-mouse IgG secondary antibody conjugated with Cy3 (Jackson Immunoresearch, West Grove, PA). After antibody incubation, oocytes were stained with DAPI (50 μg/ml) to analyse the chromatin configuration. Chromatin configuration and transcriptional activity were analysed simultaneously for each oocyte. Fluorescence was detected using a Leica TCS SP2 laser-scanning confocal microscope and the signal was quantified using NIH Image. The pixel value/unit area was measured for the nucleus, and the average value of two different regions of the cytoplasm was subtracted as background. This value was multiplied by the nuclear area to yield the total amount of fluorescence in the nucleus. Oocytes were so thin that the area reflected the volume after permeabilisation of the plasma membranes with 0.05% Triton X-100. In each experiment, the average value calculated for the nuclei in the COC-NSN group was set at 100% and the values of other groups were expressed relative to this value.

Results

Difference in the transcriptional activities of the types of fully grown GV ooctyes

No transcriptional activity was detected in SN-type oocytes irrespective of the presence of attached cumulus cells. In contrast, NSN-type oocytes were transcriptionally active. The transcriptional activity was 3 times greater in the DO/NSN group than that in the COC/NSN group. Thus, cumulus cells downregulated transcriptional activity in NSN-type oocytes (Fig. 1).

Cumulus cells and chromatin configuration affect the competence of GV oocytes to undergo GVB

Almost all SN-type oocytes underwent GVB, while some NSN-type oocytes did not (Fig. 2). The percentages of GVB in the COC/NSN and DO/NSN groups were significantly lower ($p < 0.01$) than for the SN-type oocyte groups with the corresponding cumulus cell patterns. As for SN-type oocytes, no difference was found in the frequency of GVB between the COC and

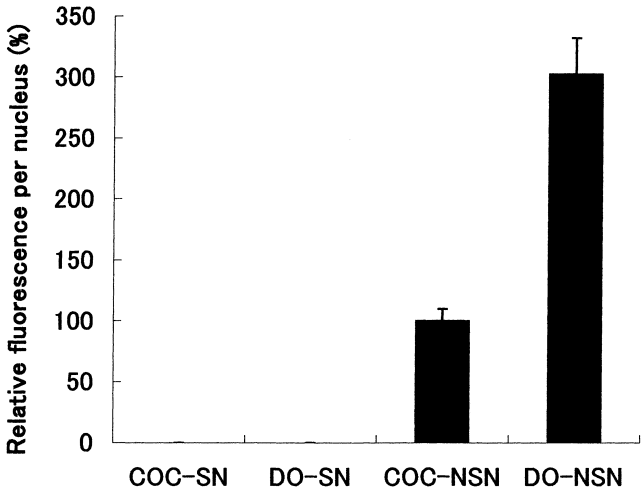


Figure 1 BrUTP incorporation in plasma-membrane permeabilised oocytes. The numbers of nuclei analysed in the COC-SN, COC-NSN, DO-SN and DO-NSN groups were 35, 42, 39 and 57, respectively. The data were accumulated from four independent experiments. The amount of BrUTP incorporated by nuclei in COC-NSN oocytes was set at 100%. COC, cumulus–oocyte complex; DO, denuded oocyte; SN, surrounded nucleolus; NSN, non-surrounded nucleolus.

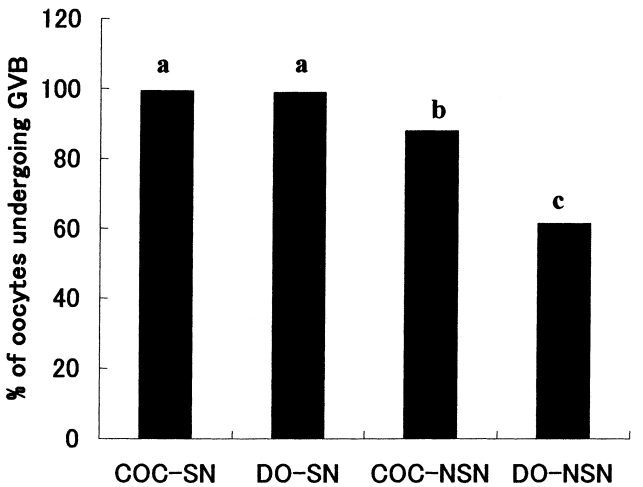


Figure 2 Comparison of GVB competence among the oocytes in various groups. Oocytes undergoing GVB were examined after 4 h of culture in maturation medium. Experiments were repeated 10 times, pooling the data from each experiment. The total number of oocytes analysed in the COC-SN, DO-SN, COC-NSN and DO-NSN groups were 422, 642, 110 and 322, respectively. Different superscripts indicate significant differences ($p < 0.01$, chi-square test). GVB, germinal vesicle breakdown; COC, cumulus–oocyte complex; DO, denuded oocyte; SN, surrounded nucleolus; NSN, non-surrounded nucleolus.

DO classes. In NSN-type oocytes, however, cumulus cells had a positive effect on the acquisition of GVB competence. The percentages of GVB were significantly higher ($p < 0.01$) in the COC/NSN group (88%) than in the DO/NSN group (61.4%).

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To exclude the possibility that the difference in the frequency of GVB was due to the difference in the sensitivity to Hoechst and UV irradiation of SN- and NSN-type oocytes, we assessed the chromatin organisation of cultured oocytes that were stained with Hoechst just before observation. Assessment of the oocytes that did not undergo GVB after 4 h of culture revealed that all the GV oocytes that failed to undergo GVB were of the NSN type.

Cumulus cells and chromatin configuration affect the competence of GV oocytes to undergo PB I extrusion

After GVB, the oocytes in all four groups progressed through the next stage of maturation. However, the percentages of the first polar body (PB I) extrusion differed. As shown in Fig. 3, the percentages of oocytes extruding PB I in the COC/SN, DO/SN, COC/NN and DO/NSN groups were 81.9%, 66.0%, 47.4% and 29.0%, respectively. SN-type chromatin configuration and association with cumulus cells were advantageous in acquiring competence to progress to the second step of meiosis.

The frequency of parthenogenetic activation differed between SN- and NSN-type oocytes

When oocytes matured *in vitro* were activated by Sr^{2+}, the frequency of parthenogenetic activation differed between SN- and NSN-type oocytes; it was 46.9% (129/275) in SN type and 27.5% (14/51) in NSN type (Fig. 4). Cumulus cells did not affect the frequency of parthenogenetic activation. No difference was found between the artificial activation rate of COC-SN (54.4%, 43/79) and DO-SN (45.2%, 70/155) types.

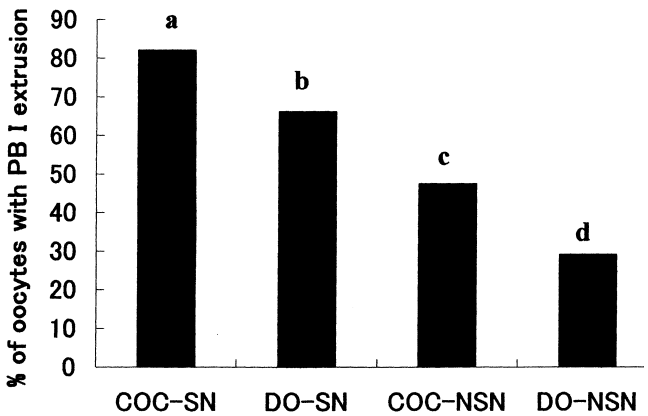


Figure 3 Comparison of competence for PB I extrusion among GVB oocytes in various groups. The percentage of GVB oocytes with PB I extruded was measured after 16 h of culture. Experiments were performed six times and the data were pooled. The total numbers of oocytes analysed in the COC-SN, DO-SN, COC-NSN and DO-NSN groups were 222, 444, 76, and 217, respectively. Different superscripts indicate significant differences ($p < 0.01$, chi-square test). GVB, germinal vesicle breakdown; PB I, the first polar body; COC, cumulus–oocyte complex; DO, denuded oocytes; SN, surrounded nucleolus; NSN, non-surrounded nucleolus.

Discussion

It has been reported that the proportions of SN- and NSN-type chromatin configurations are almost equal and are not dependent on the presence of cumulus cells or on the age of the mouse (Debey *et al.*, 1993). However, we observed that the proportions of the NSN and SN types were 27.5% and 72.5%, respectively, for cumulus–oocyte complexes, and were 40.8% and 59.2%, respectively, for denuded oocytes. It is possible that the proportion of each type of chromatin configuration varies among strains of mice.

Oocytes isolated mechanically from medium-sized or large follicles can resume meiosis spontaneously *in vitro*, and meiotic competence is acquired stepwise during oogenesis (Sorensen & Wassarman, 1976). The first step to be acquired in meiotic competence is the capacity to undergo nuclear envelope breakdown and to progress to the first meiotic metaphase (M I); this is acquired in the mouse just before or at the time of antrum formation (Sorensen & Wassarman, 1976; Wassarman & Josefowicz, 1978). The second step is the capacity to complete the progression to the second metaphase (M II). The percentage of oocytes that reach M II *in vitro* depends on the age of the mice and seems to depend on oocyte diameter (Sorensen & Wassarman, 1976; Wickramasinghe *et al.*, 1991). Oocytes from 22-day-old females that reach a diameter

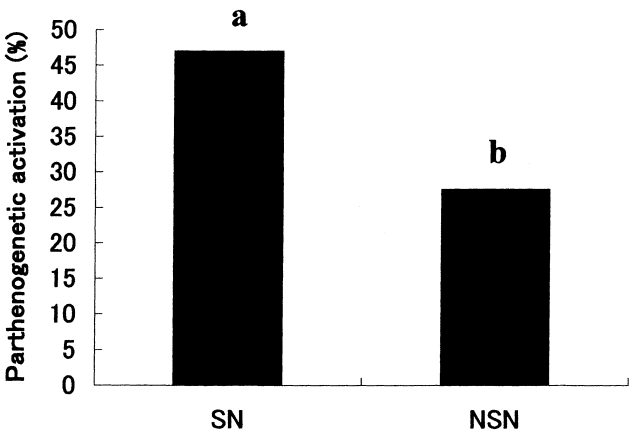


Figure 4 Comparison of the frequency of parthenogenetic activation between SN- and NSN-type oocytes. The oocytes were activated by 10 mM Sr^{2+} after 16 h of culture *in vitro*. Experiments were repeated five times and the data were pooled. The total number of SN- and NSN-type oocytes analysed was 275 and 51, respectively. Different superscripts indicate significant differences ($p < 0.01$, chi-square test). SN, surrounded nucoleolus; NSN, non-surrounded nucleolus.

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of 70 µm are fully competent to complete meiotic maturation (Eppig & Schroeder, 1989).

As for the first step of maturation, the differences in GVB competence among the four groups could be explained by the differences in transcriptional activity. Our results revealed a negative relationship between the transcriptional activity of oocytes and GVB capacity. With regard to the second step in the development of meiotic competence, it was observed that the frequency of polar body extrusion was also negatively correlated with transcriptional activity. Cumulus cells and SN-type organization had beneficial effects on acquiring meiotic competence in culture, and it was observed that these two effects were additive. The COC/SN oocyte group had the lowest transcriptional activity and the highest frequency of PB I extrusion; those of the DO/NSN group showed the opposite. In addition, it was observed that the greater the transcriptional activity, the more the meiotic capacity was impaired.

The transition from a transcriptionally active state to an inactive one is a necessary prerequisite for ovulation of a fully grown antral oocyte (De La Fuente & Eppig, 2001). In the final stage of oocyte growth there might be a mechanism responsible for the transition from a transcriptionally active to an inactive state. Oocytes escaping from this mechanism remain in a transcriptionally active state, and such abnormal transcription in NSN-type oocytes might result in abnormal components in the cytoplasm that harm the meiotic competence of the oocyte. Alternatively, transcriptionally active oocytes may still be in the process of acquiring meiotic competence. During oocyte growth and differentiation, oocytes engage in intensive RNA transcription and protein synthesis, producing products that are essential for oocyte growth and preimplantation embryo development (Rodman & Bachvarova, 1976). As growth is completed, transcription becomes silent, and sufficient molecules are stored in the oocytes for meiosis and full developmental competence. Transcriptionally active oocytes are probably still accumulating the products required for oocyte maturation and preimplantation embryo development.

Silencing of transcription was related to competence to undergo GVB, but not to competence to develop to the M II stage. In transcriptionally inactive oocytes, the frequency of PB I extrusion was improved when cumulus cells were attached. Previously, it was shown that although meiotic competence is developmentally regulated by an oocyte-intrinsic mechanism (Canipari *et al*., 1984; Chesnel *et al*., 1994), granulosa cells are also important regulators of final oocyte differentiation events (Chesnel *et al*., 1994; Chesnel & Eppig, 1995). Granulosa cells have previously been shown to regulate the post-translational modification of several murine and bovine proteins (Colonna *et al*., 1989; Cecconi *et al*., 1991; Motlik *et al*., 1996), and they are required for the completion of oocyte growth and the acquisition of cytoplasmic meiotic competence.

References

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

Bos-Mikich, A., Whittingham, D.G. & Kones, K.T. (1997). Meiotic and mitotic Ca^{2+} oscillations affect cell composition in resulting blastocysts. *Dev. Biol.* **182**, 172–9.

Bouniol-Baly, C., Hanraoui, L., Guibert, J., Beaujean, N., Szollosi, M.S. & Debey, P. (1999). Differential transcriptional activity associated with chromatin configuration in fully grown mouse germinal vesicle oocytes. *Biol. Reprod.* **60**, 580–7.

Canipari, R., Palombi, F., Riminucci, M. & Mangia, F. (1984). Early programming of maturation competence in mouse oogenesis. *Dev. Biol.* **102**, 519–24.

Cecconi, S., Tatone, C., Buccione, R., Mangia, F. & Colonna, R. (1991). Granulosa cell–oocyte interactions: the phosphorylation of specific proteins in mouse oocytes at the germinal vesicle stage is dependent upon the differentiative state of companion somatic cells. *J. Exp. Zool.* **258**, 249–54.

Chatot, C.L., Lewis, J.L., Torres, I. & Ziomek, C.A. (1990). Development of 1-cell embryos from different strains of mice in CZB medium. *Biol. Reprod.* **42**, 432–40.

Chesnel, F. & Eppig, J.J. (1995). Synthesis and accumulation $p34^{cdc2}$ and cyclin B in mouse oocytes during acquisition of competence to resume meiosis. *Mol. Reprod. Dev.* **40**, 503–8.

Chesnel, F., Wigglesworth, K. & Eppig, J.J. (1994). Acquisition of meiotic competence by denuded mouse oocytes: participation of somatic-cell products and camp. *Dev. Biol.* **161**, 285–95.

Colonna, R., Cecconi, S., Tatone, C., Mangia, F. & Buccione, R. (1989). Somatic cell–oocyte interactions in mouse oogenesis: stage-specific regulation of mouse oocyte protein phosphorylation by granulosa cells. *Dev. Biol.* **133**, 305–8.

Debey, P., Szöllösi, M.S., Szöllösi, D., Girousse, A. & Besombes, D. (1993). competent mouse oocytes isolated from antral follicles exhibit different chromatin organization and follow different maturation dynamics. *Mol. Reprod. Dev.* **36**, 59–74.

Eppig, J.J. & Schroeder, A.C. (1989). Capacity of mouse oocytes from preantral follicles to undergo embryogenesis and development of live young after growth, maturation and fertilization *in vitro*. *Biol. Reprod.* **41**, 268–76.

Eppig, J.J., Chesnel, F., Hirao, Y., O'Brien, M.J., Pendola, F.L., Waatanabe, S. & Wigglesworth, K. (1997). Oocyte control of granulosa cell development: how and why. *Hum. Reprod.* **12** (Natl. Suppl. JBFS 2), 127–32.

Fuente, R.D.L. & Eppig, J.J. (2001). Transcriptional activity of the mouse oocyte genome: companion granulosa cells modulate transcription and chromatin remodeling. *Dev. Biol.* **229**, 224–36.

Mangia, F. & Epstein, C.J. (1975). Biochemical studies of growing mouse oocytes: preparation of oocytes and analy-

https://doi.org/10.1017/S0967199402004069 Published online by Cambridge University Press

sis of glucose-6-phosphate dehydrogenase and lactate dehydrogenase activities. *Dev. Biol.* **45**, 211–20.

Mattson, B.A. & Albertini, D.F. (1990). Oogenesis: chromatin and microtubule dynamics during meiotic prophase. *Mol. Reprod. Dev.* **25**, 374–83.

Moltlik, J., Sutovsky, P., Kalous, J., Kubelka, M., Moos, J. & Schultz, R.M. (1996). Co-culture with pig membrane granulosa cells modulates the activity of cdc2 and MAP kinase in maturing cattle oocytes. *Zygote* **4**, 247–56.

Rodman, T.C. & Bachvarova, R. (1976). RNA synthesis in preovulatory mouse oocytes. *J. Cell Biol.* **70**, 251–7.

Simon, A.M., Goodenough, D.A., Li, E. & Paul, D.L. (1997). Female infertility in mice lacking connexin 37. *Nature* **385**, 525–9.

Sorensen, R.A. & Wassarman, P.M. (1976). Relationship between growth and meiotic maturation of the mouse oocyte. *Dev. Biol.* **50**, 531–6.

Swartz, W.J. & Schuetz, A.W. (1976). Morphological diversity of oocytes released from the adult mouse ovary. *Am. J. Anat.* **144**, 365–72.

Wassarman, P.M. & Josefowicz, W.J. (1978). Oocyte development in the mouse: an ultrastructural comparison of oocytes isolated at various stages of growth and meiotic competence. *J. Morphol.* **156**, 209–36.

Wickramasinghe, D., Ebert, K.M. & Albertini, D.D. (1991). Meiotic competence acquisition is associated with the appearance of M-phase characteristics in growing mouse oocytes. *Dev. Biol.* **143**, 162–72.

Whitten, W.K. (1971). Nutrient requirement for the culture of preimplantation embryos. *Adv. Biosci.* **6**, 129–39.

Worrad, D.M., Ram, P.T. & Schultz, R.M. (1994). Regulation of gene expression in the mouse oocyte and early preimplantation embryo: developmental changes in Sp1 and TATA box-binding protein, TBP. *Development* **120**, 2347–57.

Zuccotti, M., Piccinelli, A., Rossi, P.G., Garagna, S. & Redi, C.A. (1995). Chromatin organization during mouse oocyte growth. *Mol. Reprod. Dev.* **41**, 479–85.

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