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Centrosome and microtubule dynamics during early stages of meiosis in mouse oocytes

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Centrosomes, major regulatory sites for the microtubule (MT) nucleation, are regulated in a dynamic manner throughout the process of meiotic maturation. Recently, centrosome orientation in mouse oocytes has been demonstrated in metaphase I through metaphase II. However, centrosomal protein expression in concordance with MT polymerization in earlier stages of oocyte maturation from germinal vesicle stage (GV) to prometaphase I still remains unclear. The present study aims to assess the centrosome–microtubule remodelling during the onset of meiosis based on strict criteria of nuclear maturation. Six consecutive stages were determined for scoring the oocytes as unrimmed nucleolus (UR), partially rimmed nucleolus (PR), fully rimmed nucleolus (FR), nuclear lamina dissolution (NLD), disappearance of nucleolus (DON), and chromatin condensation (CC). A centrosomal protein, pericentrin, was found tightly localized adjacent to nuclear lamina in UR, lacking any MT nucleation activity. In concordance with the competency to resume meiosis, an increase in the amount and nucleation capacity of pericentrin is noted. In FR, cytoplasmic MT almost disappeared while de-novo microtubule polymerization was found in small aggregates of pericentrin localized around the nucleus. Towards the end of DON and CC, a sudden burst of pericentrin was noted with an extreme MT nucleation activity in an organized fashion that is essential for the rapid formation of first meiotic spindle. The results show that centrosomes display precisely controlled spatio-temporal changes during the onset of meiotic maturation. Accumulation of centrosomal proteins to a single locus followed by a sequestration to several spots might be evidence of a mechanism by which the proper distribution of centrosomal material during nuclear breakdown and subsequently formation of spindle are regulated in concordance with the nuclear maturation.

Key words: centrosome/chromatin/meiosis/microtubule/oocyte

Introduction

The assembly and appropriate organization of the microtubule (MT) cytoskeleton is an integral phenomenon, which is related to the expression of cellular asymmetry. Particularly in oocytes, MT display a unique paradigm as forming an eccentric meiotic spindle in a huge MT-decorated oocyte cytoplasm (ooplasm) that consequently gives rise to asymmetric cytokinesis to form the first and the second polar bodies. The driving machinery and the organization of the MT cytoskeleton are mainly confined to the centrosomes or microtubule-organizing centres (MTOC), which serve as sites of microtubule nucleation (Dictenberg *et al*., 1998). Their existence and function are dynamically regulated throughout the process of cell division, particularly during the S-phase and M-phase of the cell cycle (Khodjakov and Rieder, 1999). Briefly, centrosomes replicate and separate, most likely during prophase, followed by the nucleation and organization of the mitotic spindle MT. These centrosome dynamics have been well studied in mitosis whereas but less so in meiosis. Recently, centrosomal remodelling has been documented during the formation of meiotic spindle in mouse oocytes (Carabatsos *et al*., 2000; Combelles and Albertini, 2001). The integrity of cytoplasmic MT pattern during oocyte growth and maturation appears to be maintained by early decoration of the cytoplasmic microtubular network (CMTN). Therefore, centrosomal–microtubular orientation gains importance not only for maintaining the cytoplasmic integrity but also for the formation of asymmetrically orientated meiotic spindle. However, no direct evidence has been shown to date whether the centrosome dynamics are associated with microtubular decoration during early stages of meiosis. If changes in cytoplasmic organization do occur, the extent to which these alterations can be correlated with changes in nuclear structure and function is not known. Given the occurrence of chromatin–MT morphodynamics at specific stages of follicular development (Mattson and Albertini, 1990), the present study was designed to assess the MT reorganization with respect to the dynamic remodelling of centrosomes prior to, or onset of, the resumption of meiosis. Briefly, we demonstrate (i) the centrosomal appearance, replication, distribution and MT nucleation pattern in prophase-arrested and meiotically competent oocytes, (ii) whether the consequences of changes in chromatin organization are correlated with remodelling of the centrosomal and microtubular component of the cytoskeleton prior to and following the resumption of meiosis.

The results reveal that, in concordance with the precisely regulated activity of the nucleus as illustrated here in six consecutive stages, a sudden loss of CMTN is followed by a gradual increase both in the amount and MT nucleation activity of centrosomes when the meiotic arrest is overcome. Pericentrin protein, a well-known functional and structural marker for centrosomes (Doxsey *et al*., 1994), first appears as a non-functional solitary locus in an incompetent mouse oocyte. It then accumulates and gains microtubule nucleating activity in peripheral MTOC, and finally moves to centrally located MTOC to be involved during the formation of the meiotic spindle.

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Materials and methods

Collection, culture and fixation of oocytes

A total of 166 female Balb/c mice were reared until they reached 19–21 days old under a 12 h light–12 h dark photoperiod, ambient temperature of 21–23°C and relative humidity of 50 ± 5%. Food and water were provided *ad libitum*. Ovarian follicular development was stimulated by i.p. injection of 5 IU pregnant mare serum gonadotrophin (Sigma–Aldrich, USA). Animals were killed 48 h after injection and cumulus–oocyte complexes (COC) were collected (n = 2810) from isolated ovaries and by repeated follicular punctures. Animals and tissues were obtained by the approval and guidelines of the Institutional Ethics Committee (211-2001). Upon isolation, a group of COC were denuded from granulosa cells and were immediately fixed (T_0 oocytes) (n = 720) for the evaluation of early phases of GV oocytes. For detecting the advancement of meiotic maturation, remaining COC were cultured in single-welled IVF culture dishes for 2 h (T_2 oocytes) (n = 625) or 4 h (T_4 oocytes) (n = 805) in minimal essential medium (Sigma–Aldrich) supplemented with Earle's salts, 2 mmol/l L-glutamine, 0.23 mmol/l pyruvate, 100 IU/ml penicillin, 100 μg/ml streptomycin and 3% bovine serum albumin (BSA) in a humidified atmosphere of 5% CO_2 at 37°C. After removal of granulosa cells by gentle pipetting, oocytes were fixed and extracted for 30 min at 37°C, in a microtubule stabilization buffer (0.1 mol/l PIPES, pH 6.9, 5 mmol/l $MgCl_2{\cdot}6H_2O$, 2.5 mmol/l EGTA) containing 2% formaldehyde, 0.1% Triton X-100, 1 μmol/l taxol, 10 IU/ml aprotinin and 50% deuterium oxide (Can and Albertini, 1997), washed three times in a blocking and aldehyde reducing solution of phosphate-buffered saline (PBS) containing 2% BSA, 2% powdered milk, 2% normal goat serum, 0.1 mol/l glycine and 0.01% Triton X-100 and then stored at 4°C until processed.

Fluorescent labelling of oocytes

Oocytes were incubated in a series of primary and secondary antibodies for 2–5 h/antibody at either 4 or 37°C followed by 15 min washes in a PBS-blocking solution. Microtubules were visualized using a 1:1 mixture of α + β tubulin mouse monoclonal antibodies (1:100 dilution) (Sigma–Aldrich) followed by a 1:100 dilution of an affinity-purified fluorescein isothiocyanate goat anti-rat IgG (Jackson ImmunoResearch Laboratories, USA). Centrosomes were labelled with a polyclonal antibody directed against pericentrin (4B) (Dictenberg *et al*., 1998) at a final dilution of 1:100, followed by an affinity-purified Cy5-conjugated donkey anti-rabbit IgG (1:100 dilution) (Jackson ImmunoResearch Laboratories). For the evaluation of chromatin and chromosome staining at certain stages in meiotic progression, oocytes were dual-stained by 10 mmol/l of 7-aminoactinomycin-D (7-AA-D) (Sigma–Aldrich) (Can and Semiz, 2000) and 1 μg/ml Hoechst 33258 (Polyscience Inc., USA). Finally, oocytes were mounted on glass slides using coverslips with spacers on sides allowing an ~150 μm space in between (Can, 1996) that was filled with a 1:1 glycerol/PBS medium containing 25 mg/ml sodium azide as anti-fading reagent.

Three dimensional analyses of oocytes

Triple-labelled oocytes were initially examined by differential interference contrast (DIC) technique and a conventional fluorescence UV filter set on a Zeiss Axiovert 100M inverted microscope. Hoechst 33258 staining of nuclear material was used to verify the 7-AA-D staining (red emission). Kinetics in meiotic maturation was assessed using those two nuclear dyes.

After staging of oocytes, a Zeiss LSM-510 Meta confocal laser scanning microscope (CLSM) (Germany) equipped with ×63 plan-apo objective was used for further examination of the structural relationship of centrosomes, microtubules and chromatin. The 488 nm argon ion, 543 nm green helium–neon, 633 nm red helium–neon laser lines were used to excite microtubules, chromatin/chromosomes and centrosomes respectively. Single optical sections (1.2 μm in thickness) and serial images on z-axis at 2 μm intervals were collected by LSM-510 Software (Germany) running on a Fujitsu–Siemens workstation. The final 3-D reconstructions and volumetric measurements on 3-D images were performed using Zeiss LSM 510 3D software (Germany).

Number and volumetric measurements of pericentrin foci were statistically analysed by Kruskal–Wallis variance analysis. When the P-value indicated the significance, multiple comparison test was used (Conover, 1980) to find which groups differed from the others.

Results

Staging of oocytes due to chromatin staining patterns

Initial monitoring of the meiotic oocytes was performed by the evaluation of nuclear chromatin. All GV stage oocytes displayed a well-defined nucleus (also named as germinal vesicle) with single or occasionally two prominent nucleoli observed by nuclear fluorescent staining (Figure 1) and DIC technique (Figure 2). When oocytes were viewed using a conventional UV filter set for the visualization of Hoechst 33258 stain on nuclear chromatin, they exhibited one of six discrete patterns of chromatin organization as partly reported in mouse oocytes (Mattson and Albertini, 1990; Wickramasinghe *et al*., 1991; Bouniol-Baly *et al*., 1999). Although meiosis is considered as a continuous process, for simplicity, we refer these staining patterns based on the following criteria. The first three fluorescence patterns of chromatin organization differ with respect to the association of chromatin with the nucleolus, that is the degree of rimming of nucleolus with chromatin masses, whereas the remaining patterns depend on the major nuclear/nucleolar events. Briefly, (i) unrimmed (UR) stage, (ii) partially rimmed (PR) stage, (iii) fully rimmed (FR) stage, (iv) nuclear lamina dissolution (NLD), (v) disappearance of nucleolus (DON), and (vi) chromatin condensation (CC) (Figure 1). The nucleolus of the oocyte differs from the nucleolus of other cell types in many ways as previously reported by Kopecny *et al*. (1995) and is sometimes called the nucleolus-like body.

Frequency of oocytes at different time intervals

Oocytes displaying normal appearance (n = 2150) regarding the nucleus, ooplasm and zona pellucida were scored due to their nuclear maturation stages. Oocytes exhibiting one of any major morphological abnormalities such as pycnosis, fragmentation of ooplasm, hydration of perivitelline space or later stages of maturation (i.e. prometaphase I to metaphase II) were excluded from the study. The scoring results are summarized in Table I. The majority of cells in T_0 group which contained the COC at the time of isolation were found at UR, PR or FR stages (81%). Only a small fraction (19%) was found at later stages, a sign of the resumption of meiosis. In T_2 and T_4 groups, oocytes mostly reached NLD, DON or CC stages. Even 2 h of culture period significantly altered the percentage of maturing oocytes (25% of cells at CC stage). A constant fraction of cells remained at UR (~8%) or PR (~30%) stages even though they were cultured for 2–4 h. Interestingly, resuming cells were primarily found at either NLD or CC stages while fewer cells (~5%) were found at DON stage, implying that nucleolar disappearance is a very rapid process that exists following the dissolution of nuclear lamina during or just before the onset of chromatin condensation.

Nucleolar positioning in early stages

Both DIC observations by Nomarski optics and 3-D chromatin patterns detected by CLSM demonstrated a consistent nucleolar positioning in relation to the nuclear maturation. In all UR stage

Table I. Frequency of oocytes (n = 2810) retrieved (T_0, n = 1060) and cultured for 2 h (T_2, n = 920) or 4 h (T_4, n = 830)

Stage	T_0 (%)	T_2 (%)	T_4 (%)
Unrimmed nucleolus	14	7	9
Partially rimmed nucleolus	28	31	29
Fully rimmed nucleolus	39	4	6
Nuclear lamina dissolution	5	26	13
Disappearance of nucleolus	3	7	6
Chromatin condensation	11	25	37

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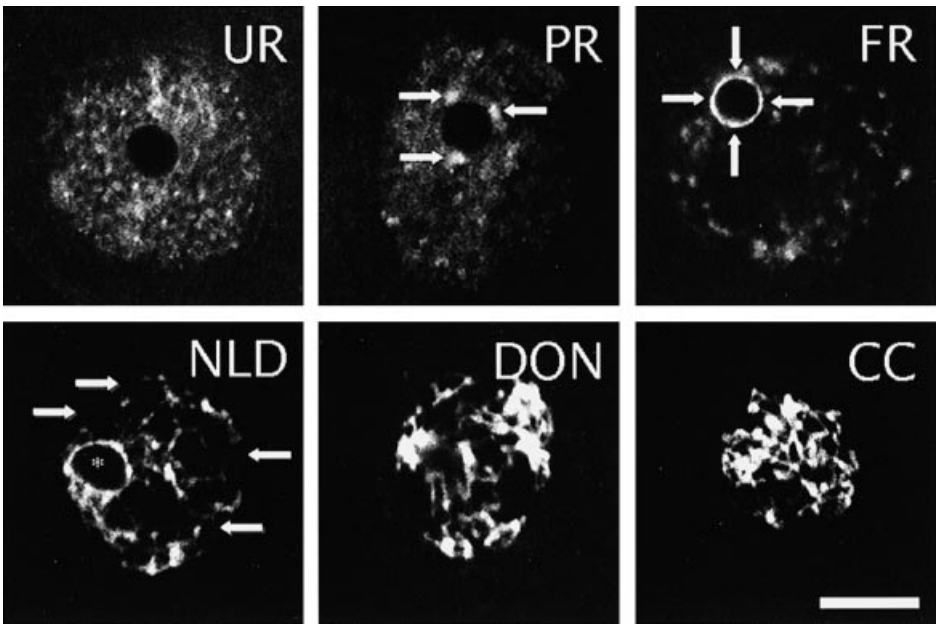

Figure 1. Nucleus, nucleolus and chromatin changes, namely nuclear maturation due to the advancement of meiosis in mouse oocytes. The unrimmed stage (UR) is characterized by the lack of any chromatin accumulation around the nucleolus. In the partially rimmed stage (PR), two to four chromatin foci (arrows) attach to the nucleolus whereas those foci increase so as to wrap the whole nucleolus (arrows) in the fully rimmed stage (FR). Dissolution of nuclear lamina from many points (arrows) stands as a dominant feature in nuclear lamina dissolution stage (NLD) followed by the immediate disappearance of the nucleolus (DON). Finally, condensation of chromatin (CC) becomes prominent as the structural constituents of nucleus and nucleolus become transiently solubilized. Scale bar = 20 μm.

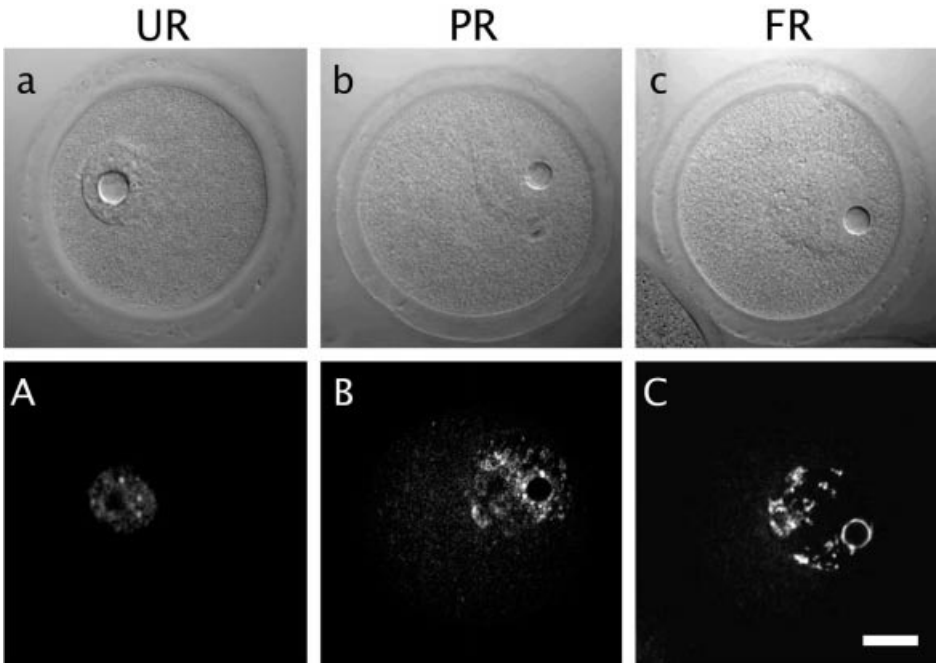

Figure 2. Translocation of nucleolus with respect to nucleus. Correlative differential interference contrast (**a**–**c**) and fluorescent images (**A**–**C**) illustrate the proximity of the nucleolus to the nuclear lamina. Initially, in the unrimmed stage (UR) stage, the nucleolus is located in the centre of the nucleus (**a**, **A**). As the oocyte matures (partially rimmed) the nucleolus shifts slightly to the peripheral karyoplasm (**b**, **B**). The nucleolus moves to a site where it associates with the nuclear lamina in the fully rimmed stage (**c**, **C**). Scale bar = 20 μm.

oocytes, the nucleolus was found at the centre of the nucleus (91.1%) (Figure 2a, A). By the onset of rimming of the nucleolus (PR stage), the nucleolus tended to translocate to the periphery of the nucleus (Figure 2b, B). More profoundly, in FR stages, the nucleolus was always found to attach to the nuclear lamina (93.6%) (Figure 2c, C).

Organization of centrosomes and microtubules

Centrosome protein pericentrin and microtubular distribution patterns corresponding to the nuclear stages were assessed by three-channel sequential detection protocol in *z*-axis. For final 3-D reconstructions, three sets of ~45 consecutive images were obtained from whole-mount

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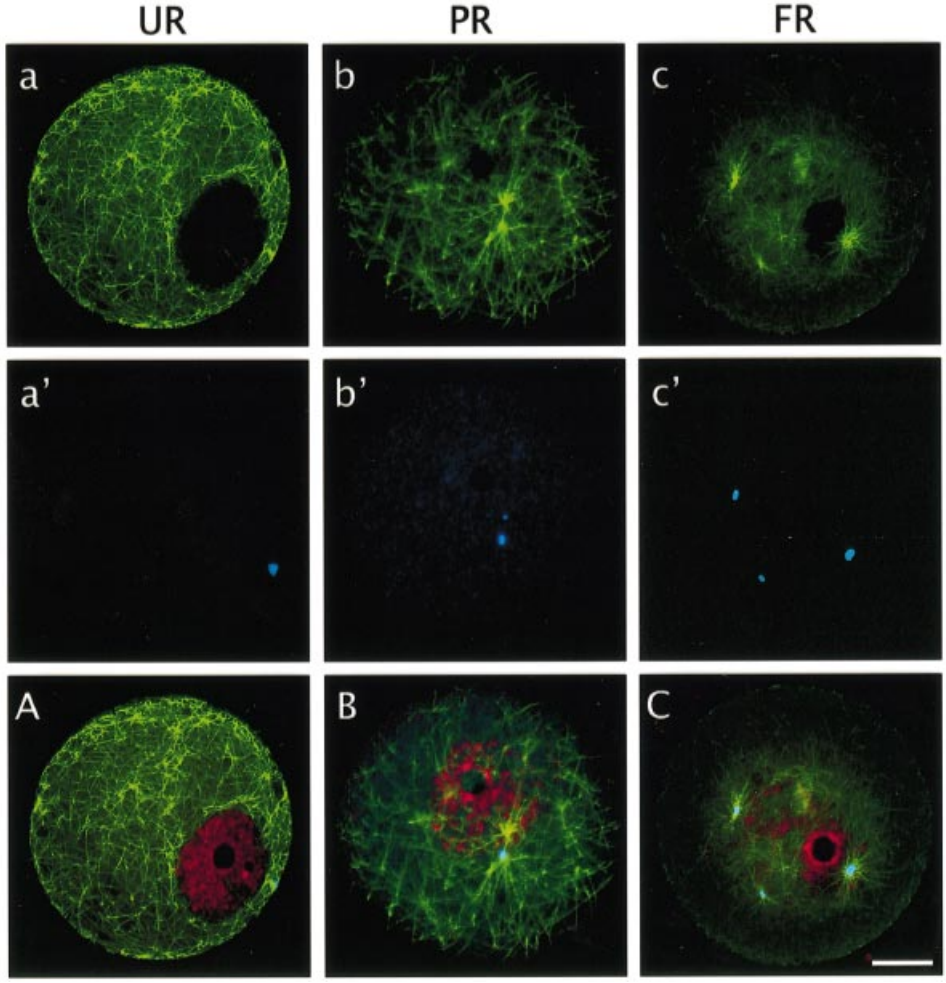

Figure 3. Three-dimensional confocal views of unrimmed (UR), partially rimmed (PR) and fully rimmed (FR) stage oocytes rendered from ~45 optical sections in *z*-axis. Micrographs in the bottom row are the overlays of the first and second rows. UR stage oocytes (**a**, **a′**, **A**) differ from other oocytes by displaying a well-decorated cytoplasmic microtubule network (CMTN). Pericentrin is restricted to a single locus typically located peripherally just beneath the oolemma as it is occasionally found adjacent to the nucleus (**A**). PR oocytes represent a relative loss of CMTN (**b**) in contrast to the increasing number of more centrally located pericentrin foci (**b′**). In FR stage, oocytes typically exhibit two to four de-novo microtubule polymerization sites (**c**) that are tightly coupled to the nucleus (**C**). Pericentrin is strictly confined to those perinuclear microtubule nucleating centrosomes (**c′**). Scale bar = 20 μm.

and pooled oocytes rather than the conventional squashed preparations (see Materials and methods).

In unrimmed (UR) stage oocytes (Figure 3A), a single (mean 1.15 ± 0.36) centrosome site was located adjacent to the cell periphery, usually just beneath the oolemma (Figure 3a′). Detection of this single centrosome locus in the *z*-axis demonstrated a condensed, small spot, irregular in nature, bearing a minimal microtubular nucleation activity (Figure 3a). No other cytoplasmic centrosome was noted at this stage. Elaborate microtubular staining was confined to CMTN throughout the ooplasm (Figure 3a).

When cells reached the next maturation stage as indicated by the appearance of chromatin rims around the nucleolus (PR stage) (Figure 3B), spatial changes did occur in microtubular and centrosomal distribution. More than one centrosome spot (mean 2.41 ± 0.6) in different sizes were usually found close to the central ooplasm (Figure 3b′). Concurrently, a reduction in CMTN was noted (Figure 3b) in association with the newly forming microtubules emerging from those centrally located centrosomes.

Remarkable changes were noted at FR stage oocytes in centrosome and microtubule organization (Figure 3C) which seem closely associated with nuclear events. The proximity of centrosomes to the nucleus in the previous stage transforms into tight contacts of a group of two to four centrosome sites (mean 3.65 ± 0.53) to the nuclear lamina and in particular, to the site where the fully rimmed nucleolus is located (95.5%) (Figure 3c, c′ and C). Compared with PR and UR stages, a remarkable increase was noted both in the volume and the number of centrosome foci at this critical stage of meiotic maturation (Figure 4A and B). In contrast to the UR and PR stages, those cytoplasmic centrosomes were found in close association with maturing nucleus at FR stage. 3-D analysis demonstrated that all centrosomes were delineated by the nuclear lamina from one side and were characterized by the de-novo polymerization of a small set of microtubules (Figure 3c and C). Simultaneously, depolymerization of CMTN, which started at PR stage, significantly increased and resulted in a total loss of CMTN (Figure 3c). 3-D-rendered FR images illustrate that the increasing MT nucleating activity of the cytoplasmic centrosomes was restricted to the deeper rather than the cortical

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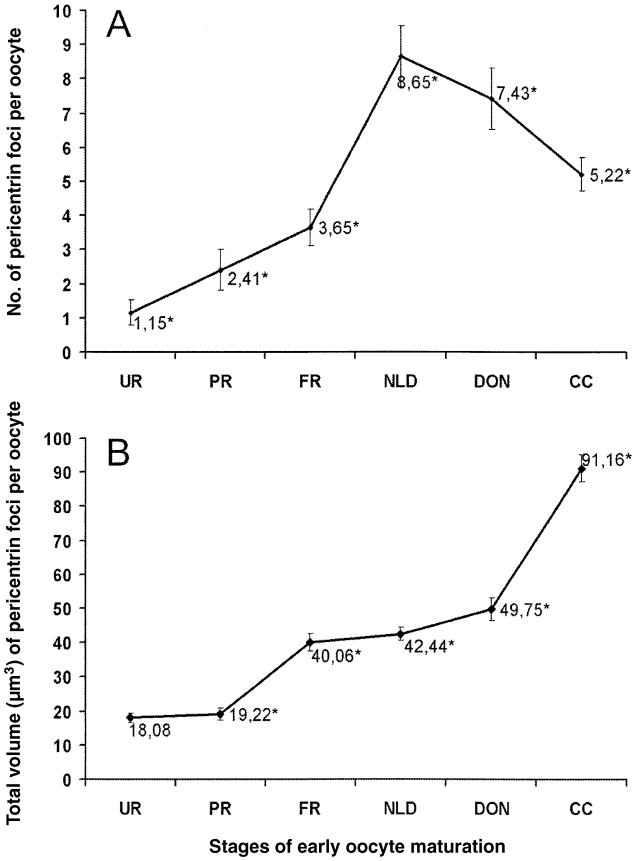

Figure 4. Number (**A**) and total volume (**B**) of pericentrin foci per oocyte throughout the stages of early oocyte maturation. Number of foci and volume measurement in μm^3 was calculated by 3-D image analysing software from single-channel *z*-series images obtained from 381 oocytes. Note the 2.2-fold increase in number of pericentrin during around nuclear lamina dissolution stage (NLD) that corresponds to the cytoplasmic scattering of the protein (**A**). While the number of foci varies throughout the stages, a remarkable increase is noted in the amount of pericentrin as the oocyte matures (**B**). Note the double-fold increase during partially rimmed (PR)–fully rimmed (FR) and disappearance of the nucleolus (DON)–condensation of chromatin (CC) transitions (**B**). The differences between each group in both **A** and **B** are statistically significant ($P < 0.05$); asterisks indicate the groups in which $P < 0.001$ when compared with the following group.

ooplasm. Therefore, it is possible to conclude that organization of the newly forming centrosomes and associated MT originates from the inner cytoplasm, possibly by the close contact to the dynamic nuclear and nucleolar events.

During the culture period (2–4 h), oocytes reached the NLD, DON or CC stages (see Table I for frequencies). A total of 26% of oocytes were detected at NLD stage during which the nuclear lamina dissolves from many points (Figure 1 and Figure 5A). In concert with this, a rapid change in centrosomal redistribution was noted in ooplasm. Many peripherally located centrosomes (mean 8.65 ± 0.88) emerged (Figure 5a, a′ and A) in addition to the centrally located ones albeit in a lesser quantity. Due to the varying number and size of centrosomes (Figure 5a′, Figure 4A and B), MT originating from these centrosomes also varied in number and length (Figure 5a).

A relatively small number of oocytes (13%) was detected in the DON stage when the nucleolus starts to degenerate (Figure 1 and Figure 5B). No major difference was noted compared to the previous stage regarding the centrosomal and microtubule distribution. Cortical and central centrosomes maintained their integrity and number with a slight increase in microtubule nucleation (Figure 5b). Quantitative measurements showed that the mean number of centrosomes slightly decreased (16%, mean 7.43 ± 0.9; Figure 4A) while the total volume of centrosomes increased 17% (Figure 4B) compared with NLD stage. As a result, oocytes reached a state where numerous cytoplasmic centrosomes nucleate MT and were distributed homogeneously throughout the ooplasm.

Finally, just before the meiotic spindle is formed, cells reached the CC stage, which was characterized by the thickening and condensation of chromatin (Figure 1 and Figure 5C). A burst of microtubule polymerization was noted, presumably due to the increased amount (Figure 4B) and nucleating capacity of centrosomes (Figure 5c'). Interestingly, major centrosome sites (mean 5.22 ± 0.49) were located in close contact with condensing chromosomes demonstrating a huge mass of centrally located microtubular array (Figure 5c and C). The latter organization will eventually transform into a monopolar, then a bipolar spindle in prometaphase and metaphase stages respectively.

Figure 6 summarizes the accumulated data regarding the centrosomal, microtubular and nuclear remodelling of proteins from unrimmed GV to the end of CC stage obtained in several experiments. It is apparent that there is a spatio-temporal interrelation between centrosomes, MT nucleation and nuclear activity, a critical period during which protein synthesis persists, most likely to build the dividing machinery of meiotic oocytes.

Discussion

The present studies extend earlier observations (Maro *et al*., 1985; Schatten *et al*., 1986; Messinger and Albertini, 1991; Wickramasinghe and Albertini, 1992; Carabatsos *et al*., 2000) on the organization of centrosomes with regard to the assembly of central and peripheral MT in mouse oocytes. We demonstrate that a constitutive centrosomal protein, pericentrin, is exclusively and dynamically assembled into cytoplasmic small aggregates during the early stages of meiosis. Recently, we reported that pericentrin is primarily confined to spindle poles during the subsequent stages of meiosis I and II (A.Can, O.Semiz and O.Çinar, submitted for publication). Together with previous findings reported by Carabatsos *et al*. (2000) and Combelles *et al*. (2001), the reorganization of major centrosomal proteins (i.e. pericentrin and γ-tubulin) and microtubule cytosleketon has been now fully documented in mouse oocytes. Growing evidence emerges from the present work and other studies that changes in oocyte nuclear and cytoplasmic organization are accomplished with a high spatio-temporal accuracy to ensure the progression of peri- and postfertilization events on time and without error. The fact that multiple centrosomes exist in oocytes and that they are dynamically regulated with respect to location and cell cycle state (Carabatsos *et al*., 2000) implies that, as a whole, the oocyte centrosome complex serves to sort and coordinate the multiple activities of nuclear and cytoplasmic maturation.

The localization of another major centrosomal protein, γ-tubulin, to condensing chromatin in insect oocytes seems to preclude direct requirements for female centrosomes (Wolf and Joshi, 1996). Studies on γ-tubulin distribution in mouse oocytes undergoing meiotic maturation showed that γ-tubulin is associated with both meiotic spindle and cytoplasmic centrosomes (Combelles and Albertini, 2001). Coupled with these findings, our data suggest that oocyte centrosomes undergo changes in their MT nucleation capacity during meiotic cell cycle progression, due in part to a loss or gain of pericentrin and γ-tubulin.

While spindle pole centrosomes are not unexpected participants in the process of meiosis, the dynamics of cytoplasmic centrosome organization have remained confusing. Earlier reports on the existence

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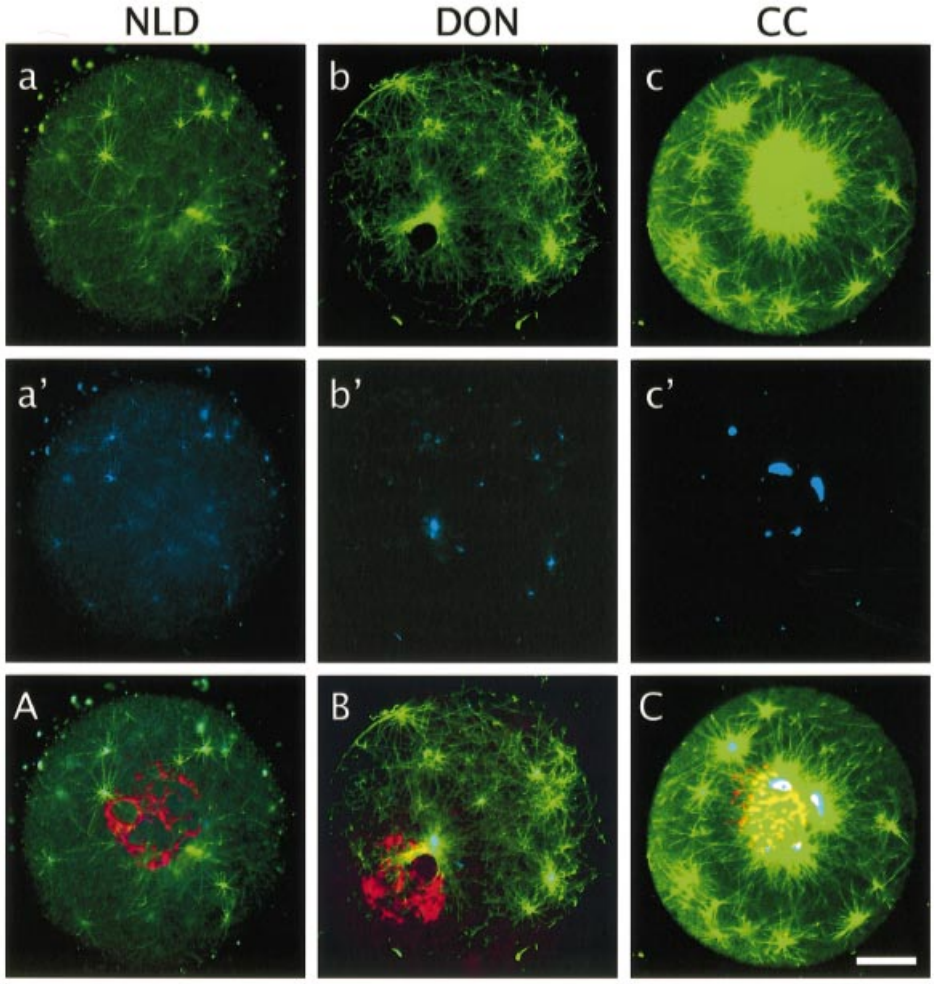

Figure 5. Three-dimensional confocal views of nuclear lamina dissolution (NLD), disappearance of the nucleolus (DON) and condensation of chromatin (CC) stage oocytes rendered from ~45 optical sections in *z*-axis. Micrographs at the bottom row are the overlays of the first and second rows. Oocytes at NLD stage is characterized by the scattering and increasing number of pericentrin (**a′**) and by radiating microtubules both in central and peripheral cytoplasm (**a** and **A**). No significant change occurs (**b**, **b′** and **B**) as cells proceed to the next stage when nucleolus degenerates (DON). Pericentrin persists at both central and peripheral centrosomes (**b′**). Prior to prometaphase I, when cells reach to CC stage an abrupt increase is often noted in the ooplasm regarding the microtubule nucleation activity (**c**). Asters of microtubules are found both in central and peripheral ooplasm (**c** and **C**). However, pericentrin tends to reaccumulate in the central ooplasm (**c′**) and reaches to its highest amount among other stages (see also Figure 4). Scale bar = 20 μm.

of these structures in mature metaphase II mouse oocytes suggested that they represent a maternal store of centrosomes that supports early embryonic cleavages (Maro *et al*., 1985; Schatten *et al*., 1986). Consistent with this is the notion that mice and other rodents represent oddity in the general scheme of centrosome inheritance, since paternal centrosomes are not involved in the formation of zygotic mitotic spindles (Schatten, 1994). Other than in the case of rodents (Maro *et al*., 1985; Albertini, 1987; Plancha and Albertini, 1994), cytoplasmic centrosomes have not been observed in freshly isolated or in cultured oocytes. Thus, studies exploring the role of cytoplasmic centrosomes or species representation lend credence to their dynamics or relative contributions to meiosis and embryonic development. Given that mouse oocytes possess central and peripheral centrosomes depending on the cell cycle stage, altered positioning and/or nucleation capacity of centrosomes associated with aberrant MT organization is likely to result in chromosome segregation abnormality and defective cytokinesis. Similar data obtained in this study from hundreds of mouse oocytes indicate that temporal microtubule organization regulated by centrosomes during meiotic maturation is a series of events in which variations or errors are extremely rare, at least in mice. On the other hand, human oocytes were reported to have a tendency to build cytoplasmic centrosomes only when treated with a high dose (10 μmol/l for 20 min) of taxol, a drug which forces the soluble tubulin to polymerize into MT (Battaglia *et al*, 1996). Temporal appearance of those cortically oriented human centrosomes coincides with the maturation process. No cytoplasmic centrosomes were found in prometaphase I, even in the presence of taxol, whereas many of them appeared when cells were at MI or MII. Therefore, it seems likely that oocytes in general display plasticity in forming centrosomal or cortical centrosomes, presumably depending on their state of maturation since they possess the complex machinery to polymerize cytoplasmic microtubules and to distribute the cargo molecules even during the ongoing cell division process. Van Blerkom (1991) speculated that the mouse oocyte uses MTOC domains to relocate mitochondria into regions requiring high ATP concentrations due to high metabolic demands (e.g. spindle assembly after GV). Since intracellular ATP concentrations are important to the viability of an oocyte (Van Blerkom *et al*., 1995), it is possible that some of the cytoplasmic MTOC may help with mitochondrial location.

Maturation scores demonstrate that total percentage of unrimmed and partially rimmed stage oocytes remained relatively constant (38–

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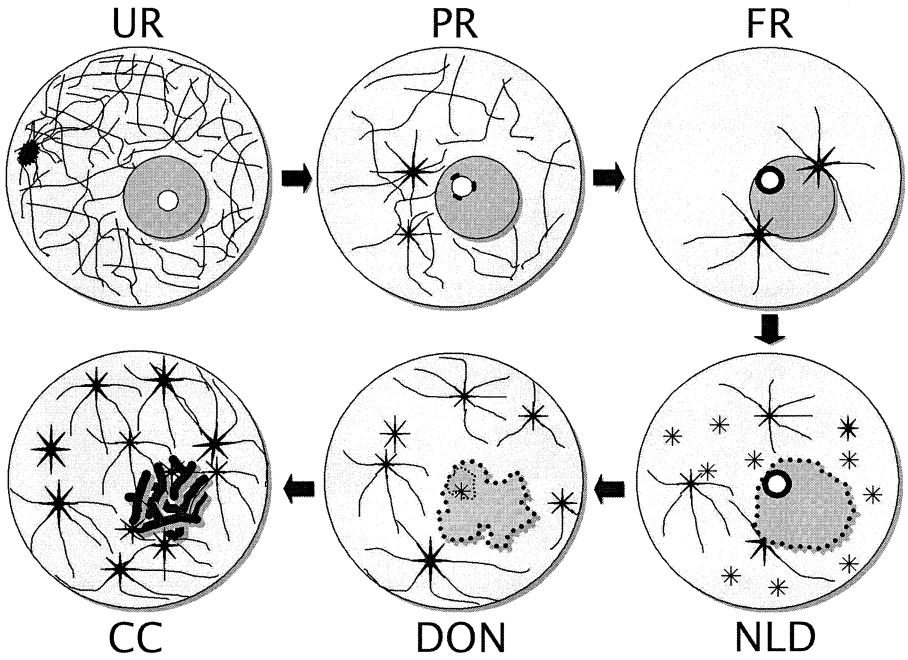

Figure 6. Summary of microtubule (MT)–centrosome–nucleus–nucleolus dynamics during the early stages of oocyte maturation based on the cumulated data from present and earlier studies. Cells undergo a profound alteration particularly in MT decoration even in a 2–4 h culture period. MT polymerization adjacent to the nucleus prompts the immediate depolymerization of cortical MT, at times when demands for MT patterning shift from stabilization of the cortex to morphogenesis of spindle.

42%) throughout the culture periods. In contrast, the remaining cells advanced to the later stages in culture conditions, suggesting that unrimmed and partially rimmed oocytes either undergo a slower rate of maturation or are not able to resume maturation, at least in in-vitro conditions, as also reported by Wickramasinghe and Albertini (1992) and Debey *et al*. (1993). In contrast, fully rimmed and the later stages of oocytes appeared to advance in maturation and mostly reach chromatin condensation stage. Differences in percentage of cells among FR and NLD stages implies that each stage has different length, therefore the lower frequency of cells detected during the DON stage might be due to shorter duration of this stage or to the faster progress of oocytes that fulfil the requirements to advance to the next stage.

In the present study, the detailed monitoring of the differences in chromatin configuration in six distinctive steps, which have not been described previously, helped us to follow the synchronous events in ooplasm, particularly the redistribution of pericentrin. Upon acquiring sudden changes in the amount of pericentrin and MT nucleation activity, we performed a quantitative analysis of immunodetectable pericentrin to understand whether the cessation of RNA transcriptional activity (Miyara *et al*., 2003) and the protein synthesis during that period coincides with the remodelling of pericentrin. Our results demonstrate that the number of pericentrin foci is variable during nuclear maturation so as to balance the microtubule polymerization activity between central and peripheral ooplasm. In contrast, the amount of pericentrin consistently increases (5.1-fold) during the stages measured (from UR to CC). This gradual increase in the amount of the protein as opposed to the changing number of polymerized form, and the significantly higher variation coefficient in the number at each stage (compare SD of Figure 4A and B), indicate that increased pericentrin expression that occurs most likely to cope with the increasing requirement for microtubule nucleation and organisation does not necessarily correspond to the number of pericentrin foci. This discordance may also indicate that pericentrin expression and distribution are regulated by different factors whose primary functions are differentially expressed within the cell. Accumulating large pools of centrosomal proteins such as pericentrin and γ-tubulin (Combelles and Albertini, 2001) is an expected consequence of the growth phase of oogenesis given the need to support centrosome functions in embryonic mitoses, independent of parental inheritance patterns (Schatten, 1994). The gradual and substantial increase of pericentrin as a major centrosomal protein during the onset of meiosis reconciles the previous reports emphasizing the importance of protein synthesis during these stages for a successful gametogenesis and fertilization (Gosden *et al*., 2003). Because animal oocytes do not possess centrioles (Szollosi *et al*., 1972; Maro *et al*., 1985), the oocyte centrosome(s) must contain all the biochemical components necessary to mediate the polymerization of tubulin dimers into microtubules.

Bouniol-Baly *et al*. (1999) demonstrated that unrimmed and partially rimmed oocytes are transcriptinally active whereas RNA transcription activity ceases when cells reach the fully rimmed stage. The transcriptional activity drops to zero as soon as condensed chromatin begins to wrap around the nucleolus. Furthermore, transcriptional capacity of an oocyte has been shown to relate to the meiotic competence (Fair *et al*., 1997). The fact that higher chromatin condensation level and transcriptional inactivity are always associated in fully rimmed mouse oocytes (Bouniol-Baly *et al*., 1999) suggests a biochemical link between these two factors. A likely candidate could be the M-phase specific $p34^{cdc2}$ kinase/cyclin B (maturation promoting factor; MPF), as fully rimmed GV oocytes present M-phase characteristics, particularly in terms of microtubule assembly (Wickramasinghe *et al*., 1991). Disappearance of the nucleolus is known to be regulated by MPF via the phosphorylation of nucleolin (Belenguer at al., 1990) and other nucleolar components. Concomitant with chromatin condensation, the nuclear lamina breaks down and the lamin proteins comprising the nuclear lamina become hyperphosphorylated (Heald and McKeon, 1990). Taken together, both functional and structural coordination of nuclear and nucleolar

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events for the remodelling maturing oocytes follow a series of consecutive events which determines the competency of a given oocyte through a mechanism orchestrated by MPF.

Conclusively, as the success rate of fertilization and the quality of the embryo following fertilization are primarily determined by the oocyte itself, and more specifically, important cell cycle regulators are localized to the oocyte meiotic spindle (Kubiak *et al*., 1992), mounting evidence suggests that major proteins of cell division machinery such as pericentrin, α-, β- and γ-tubulin, motor protein dynein, and chromatin serve as quantitative and qualitative determinants of meiotic spindle formation and function. Although mouse-specific variations may not be extended to other species, evidence obtained from other cell types and from mitosis suggests that cell cycle regulators and related constitutive proteins are structurally and functionally similar. Therefore, one can postulate that errors in those regulatory pathways can give rise to aneuploidy, fertilization failure and embryo loss. Apparently, correlative studies in human oocytes are needed to answer the questions regarding the major determinants of oocyte quality especially during its final stage of maturation prior to fertilization.

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