

Original Article

Dynamics of Lamins B and A/C and Nucleoporin Nup160 during Meiotic Maturation in Mouse Oocytes

(oocytes / meiosis / meiotic spindle / nuclear lamina / Nup107-160 / nuclear pore complex)

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Abstract. This study was aimed at elucidating the fate of three important nuclear envelope components – lamins B and A/C and nucleoporin Nup160, during meiotic maturation of mouse oocytes. These proteins were localized by epifluorescence and confocal microscopy using specific antibodies in oocytes at different stages from prophase I (germinal vesicle) to metaphase II. In immature germinal vesicle oocytes, all three proteins were detected at the nuclear periphery. In metaphase I and metaphase II, lamin B co-localized with the meiotic spindle, lamin A/C was found in a diffuse halo surrounding the spindle and to a lesser degree throughout the cytoplasm, and Nup160 was concentrated to the spindle poles. To our knowledge, this is the first report on nucleoporin localization in mammalian oocytes and the first successful detection of lamins in mature oocytes. While the distribution patterns of both lamins closely paralleled the respective stages of mitosis, Nup160 localization in metaphase oocytes corresponded to that in mitotic prometaphase rather than metaphase. The peculiar distribution of this nucleoporin in oocytes may reflect its role in meiosis-specific mechanisms of spindle assembly and its regulation.

Introduction

The developmental potential of the zygote depends on successful rearrangement of chromatin during oocyte meiotic maturation. It is mediated by extremely com-

plex reorganization of the cytoskeleton and nuclear envelope (Delimitreva et al., 2012). Although the early meiotic stages have been relatively well studied, the events of final steps of oocyte meiosis (from meiotic resumption in late prophase I until metaphase II) are still poorly understood. The oocyte nucleus in late prophase I, traditionally called GV (germinal vesicle), becomes competent to resume meiosis upon accumulation of pericentriolar heterochromatin called karyosphere, surrounding nucleolus or rimmed nucleolus (Can et al., 2003; De la Fuente et al., 2004; Tan et al., 2009). Then, the nucleus disaggregates in the so-called germinal vesicle breakdown (GVBD) stage. A functional nuclear envelope will be reconstituted late in the pronuclear zygote, if fertilization occurs. However, the fate of nuclear envelope components between GVBD and zygote stage is still unclear. Tracing them is necessary in the light of what is known about their function in other cell types, not only in the intact nucleus but also during cell division.

The major nuclear envelope proteins are lamins and nucleoporins. The innermost layer of nuclear envelope responsible for its mechanical strength is the nuclear lamina composed of B and A/C lamins (Dechat et al., 2008), while the nuclear pores allowing nucleocytoplasmic transport of macromolecules are supported by the nuclear pore complex composed of nucleoporins, among which a prominent role is played by complex Nup107-160 (Orjalo et al., 2006). During mitosis, both the lamina and the nuclear pore complex undergo specific redistribution concomitant with nuclear envelope breakdown and mitotic spindle formation. At the G2/M transition, activated maturation promoting factor p34cdc2 causes phosphorylation of lamins. This leads to dissociation of lamin A/C from the inner nuclear membrane and its dispersal throughout the cytoplasm. Lamin B, which is farnesylated, remains attached to the membrane but can no longer provide support to it, resulting in nuclear envelope breakdown into small vesicles (Dechat et al., 2008). These vesicles tend to remain in the vicinity of the mitotic spindle and to overlay it, forming the so-called lamin B spindle matrix (Tsai et al., 2006). After the metaphase-anaphase transition, protein phos-

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Abbreviations: BSA – bovine serum albumin, FITC – fluorescein isothiocyanate, FSH – follicle-stimulating hormone, GV – germinal vesicle, GVBD – germinal vesicle breakdown, IU – international units, PBS – phosphate-buffered saline, TRITC – tetramethylrhodamine isothiocyanate.

phatases dephosphorylate lamins, allowing nuclear envelope reassembly (Dechat et al., 2008).

The nuclear pore complex also disassembles during mitosis, but individual nucleoporins forming Nup107-160 remain associated (Loiodice et al., 2004). During prometaphase, they co-localize first with spindle poles and kinetochores and then with proximal spindle fibres. By metaphase, however, Nup107-160 detaches from microtubules and disperses throughout the cytoplasm, where it remains until late anaphase (Orjalo et al., 2006; Gütinger et al., 2009). Nup107-160 is not a passive spindle pole component but is required for mitotic spindle assembly, as shown by depletion experiments (Orjalo et al., 2006).

With regard to the important role of these nuclear proteins, their presence and localization in the oocyte is of considerable interest. Mutations in genes encoding Nup107-160 components have recently been found to disrupt human ovarian development (Weinberg-Shukron et al., 2015). However, to our knowledge, there are no published data about the localization of Nup107-160 components during mammalian oogenesis, and insufficient data about the lamin localization. Both B and A/C lamins have been detected in the nuclear envelope of prophase-arrested oocytes in germinal vesicle stage (Schatten et al., 1985; Prather et al., 1989). They form a well-developed nuclear lamina (Can et al., 2003). While B lamins are known to be more universal and widely distributed than the A/C lamins with their tissue-specific expression, some authors report smaller quantity and more variable distribution of B lamins in oocyte nuclei, compared to A/C lamins (Hall et al., 2005; Foster et al., 2007).

Meiotic resumption is associated with changes in the intracellular distribution of oocyte lamins. At germinal vesicle breakdown stage, lamin A/C may form an aggregate of irregular shape in the vicinity of chromosomes (Prentice-Biensch et al., 2012). Sanfins et al. (2004), detecting lamin B in maturing mouse oocytes, describe an initial collapse of the nuclear lamina, followed by gradual expansion of lamin B-reactive structures in prometaphase. However, because their work primarily addresses maturation conditions, lamin B localization does not extend to metaphase I and II stages. Other authors report disappearance of both B and A/C lamins in metaphase I and mature metaphase II oocytes (Prather et al., 1989; Prentice-Biensch et al., 2012). In a recent study, lamin A/C was detected around the metaphase plate in metaphase I oocytes but disappeared after cytokinesis (Susor et al., 2015). However, during fertilization, nuclear lamina with typical structure and composition containing both types of lamins has been described in the pronuclear zygote (Prather et al., 1989; Foster et al., 2007; Kelly et al., 2010).

Our recent preliminary study has shown lamin B to be present and associated with meiotic spindle in metaphase mouse oocytes (Nikolova et al., 2015). To clarify in more detail the fate of lamins during oocyte maturation and to elucidate the presence and dynamics of

Nup107-160, we performed an immunocytochemical study of B and A/C lamins and nucleoporin Nup160 in mouse oocytes at stages from GV to metaphase II.

Material and Methods

Prepubertal (4–5 weeks old) virgin female mice, strain BALB/c, were obtained from the breeding facility of the Medical University of Sofia. All experiments with them were planned in accordance with EU and Bulgarian legislature and approved beforehand by the Medical University of Sofia Research Ethics Commission.

Part of the animals were subjected to ovarian stimulation by 5 IU Menogon (Ferring, Kiel, Germany) followed by 5 IU Pregnyl (Organon, Oss, Netherlands) 48 h later, administered by intraperitoneal injection on a one-time basis for each hormone. The mice were euthanized by cervical translocation under anaesthesia 16 h after the second injection. Meiotically matured (ovulated) and immature oocytes were retrieved from the oviductal ampulae and the ovaries, respectively. They were washed in clean Leibowitz medium (Sigma-Aldrich, Darmstadt, Germany) and briefly treated with 0.5 mg/ml hyaluronidase (Sigma-Aldrich) in the same medium to remove cumulus cells. After that, oocytes were fixed for 45–60 min in 2% paraformaldehyde and 0.04% Triton X-100 dissolved in phosphate-buffered saline (PBS), pH 7.2.

The rest of the mice were injected with Menogon alone and euthanized 40 h later. Their ovaries were placed in an embryological Petri dish with Leibowitz medium. Immature oocytes were isolated by puncturing antral follicles. They were cultured for *in vitro* maturation in a Hera cell 150 incubator (Heraeus, Hanau, Germany) for 24 h at 37 °C and 5% CO₂ in α MEM medium (Sigma-Aldrich) supplemented with 50 μ g/ml ascorbic acid, 10 μ g/ml transferrin, 5 μ g/ml insulin, 5% foetal calf serum, 10 IU/ml penicillin/0.01 mg/ml streptomycin (Sigma-Aldrich) and 0.075 IU/ml FSH (Serono, Modugno Bari, Italy). After that, oocytes were fixed similarly as those obtained by ovarian stimulation.

Indirect immunofluorescence was performed as described before (Delimitreva et al., 2006). Lamin B, lamin A/C and Nup 160 were detected by polyclonal rabbit anti-lamin B2 antibody, monoclonal mouse anti-lamin A/C antibody (clone No. 4C11) and polyclonal rabbit anti-nucleoporin 160 antibody, respectively. All these primary antibodies were products of Sigma-Aldrich and were diluted 1 : 200 in PBS (pH 7.2) with 0.3% bovine serum albumin (BSA) and 1% sodium azide (Sigma-Aldrich). Apart from the nuclear proteins, α -tubulin was localized by monoclonal mouse anti- α -tubulin antibody, clone DM1A (Sigma-Aldrich) diluted 1 : 1000 in PBS (pH 7.2) with 0.3% BSA and 1% sodium azide. In some experiments, co-localization of lamin B or Nup160 with α -tubulin and of lamin A/C with Nup160 was studied by applying the respective first antibodies together.

After incubation with the primary antibodies and washing, oocytes were transferred to secondary antibody

solution. Mouse primary antibodies were visualized by FITC-conjugated anti-mouse IgG (Sigma-Aldrich) diluted 1 : 200 and rabbit primary antibodies by TRITC-conjugated anti-rabbit IgG (Sigma-Aldrich) diluted 1 : 500 in the same PBS-BSA buffer. The secondary antibody solution also contained 10 µg/ml Hoechst 33258 (Sigma-Aldrich) for visualization of DNA. After washing, oocytes were mounted in polyvinyl alcohol (Fluka, Münster, Germany) and transferred to microscopic slides. All oocytes were observed by epifluorescent microscope Axioskop 200 (Zeiss, Göttingen, Germany) and later some of them were also subjected to laser scanning confocal microscopy using a Leica TSC SPE (Leica Microsystems, Wetzlar, Germany) microscope.

Results

Oocytes in early GV stage were characterized by diffuse chromatin appearance and absence of karyosphere. At this stage, all three nuclear proteins were localized at the periphery of the nucleus, corresponding to the location of its envelope (shown for lamin A/C and Nup160 in Fig. 1 A-C).

Prophase oocytes that had started to mature could be recognized by the presence of karyosphere (surrounded nucleolus) as an intranuclear spherical structure of condensed chromatin. After formation of karyosphere, lamin A/C and Nup 160 but not lamin B associated with its periphery. At this stage, the reaction for these two proteins delineated two concentric spheres corresponding to the nuclear envelope and the karyosphere, respectively (Fig. 1 D-F). The inside of the karyosphere did not show reaction with antibodies used in this study.

In late GV and GVBD, gradual chromatin condensation could be observed. Condensed chromatin detached from the nuclear envelope relatively early, while the chromatin rim of the karyosphere persisted for a longer time. Deformations of the nuclear envelope reflecting its breakdown were revealed by the reaction for lamins B and A/C. Nup160 dissociated from the nuclear periphery and was found throughout the nuclear volume, with the exception of the karyosphere interior. α -Tubulin, previously found only in the cytoplasm, at this stage was also detected in the nucleus (Fig. 1 G-L).

During metaphase I and metaphase II, chromatin staining revealed shortened compact chromosomes organized in metaphase plates. None of the studied nuclear proteins co-localized with the condensed chromosomes, but all three showed a pattern of association with the spindle. This association was weakest for lamin A/C, which was detected throughout the cytoplasm, with a brighter reaction surrounding the spindle as a diffuse halo without binding to the fibres themselves (Fig. 2 A, D, G). Lamin B showed co-localization with the meiotic spindle. The co-localization was full and extended along the entire length of the spindle fibres (Fig. 2H shows labelling for lamin B and Fig. 2I for α -tubulin in the same cell). The lamin B reaction had a fine granular appearance. Nup160 was also associated with the spindle,

but in a different way, being concentrated at the spindle poles (Fig. 2 B, E, G, J; specifically co-localized with α -tubulin in Fig. 2 J-K). From a suitable angle of observation, the reaction for Nup160 was in the shape of rings corresponding to the poles (Fig. 2 B, J).

All studied proteins showed apparently identical distribution patterns in metaphase I and metaphase II. No difference was noted between oocytes obtained by ovarian stimulation and those obtained by *in vitro* maturation.

Discussion

The aim of the present study was to localize lamins B and A/C and Nup160 (member of the Nup107-160 nucleoporin complex) in mouse oocytes at different maturation stages and to compare the findings to the known distribution of these proteins in somatic cells at different stages of the mitotic cell cycle. To our knowledge, the current literature contains no studies of nucleoporin localization in oocyte meiosis and no reports detecting lamin B or A/C in metaphase oocytes, with the exception of a recent work detecting lamin A/C in metaphase I (Susor et al., 2015) and our preliminary data for lamin B (Nikolova et al., 2015).

In prophase-arrested oocytes (early GV stage), the reaction for both types of lamins was restricted to the nuclear envelope as in somatic cells and as other authors have reported for oocytes at this stage (Schatten et al., 1985; Prather et al., 1989). The localization of Nup160 to the same domain was also expected. However, after karyosphere formation, partial redistribution of lamin A/C and Nup160 to it was observed. The association of lamin A/C and Nup160 with the karyosphere shows interaction of these proteins with condensed chromatin not only in the nuclear periphery, where lamin-chromatin interactions have been known for a long time (Dechat et al., 2008), but also inside the nucleus. The shifting to the karyosphere or “surrounded nucleolus” has no known analogue in the mitotic cell cycle; however, since this structure differs in important respects from the nucleolus of somatic cells, it is expected to show unique immunocytochemical characteristics. The apparent absence of lamin B relocation to the karyosphere could be explained by anchoring of this protein to the inner nuclear membrane.

In late GV stage and at the transition to GVBD, three changes in the intracellular localization of the studied proteins were observed: dissociation of Nup160 from the nuclear periphery, deformation of the nuclear lamina revealed by the reaction for lamins, and detection of tubulin inside the nucleus. All three changes seemed to reflect alterations of the nuclear envelope structure preparing it for complete breakdown. They coincided with chromatin changes, namely, overall condensation and detachment from the envelope, removing the visible reaction for chromatin from the peripheral nuclear region. In other words, the imminent disappearance of the nucleus as a cell compartment was preceded by relocation

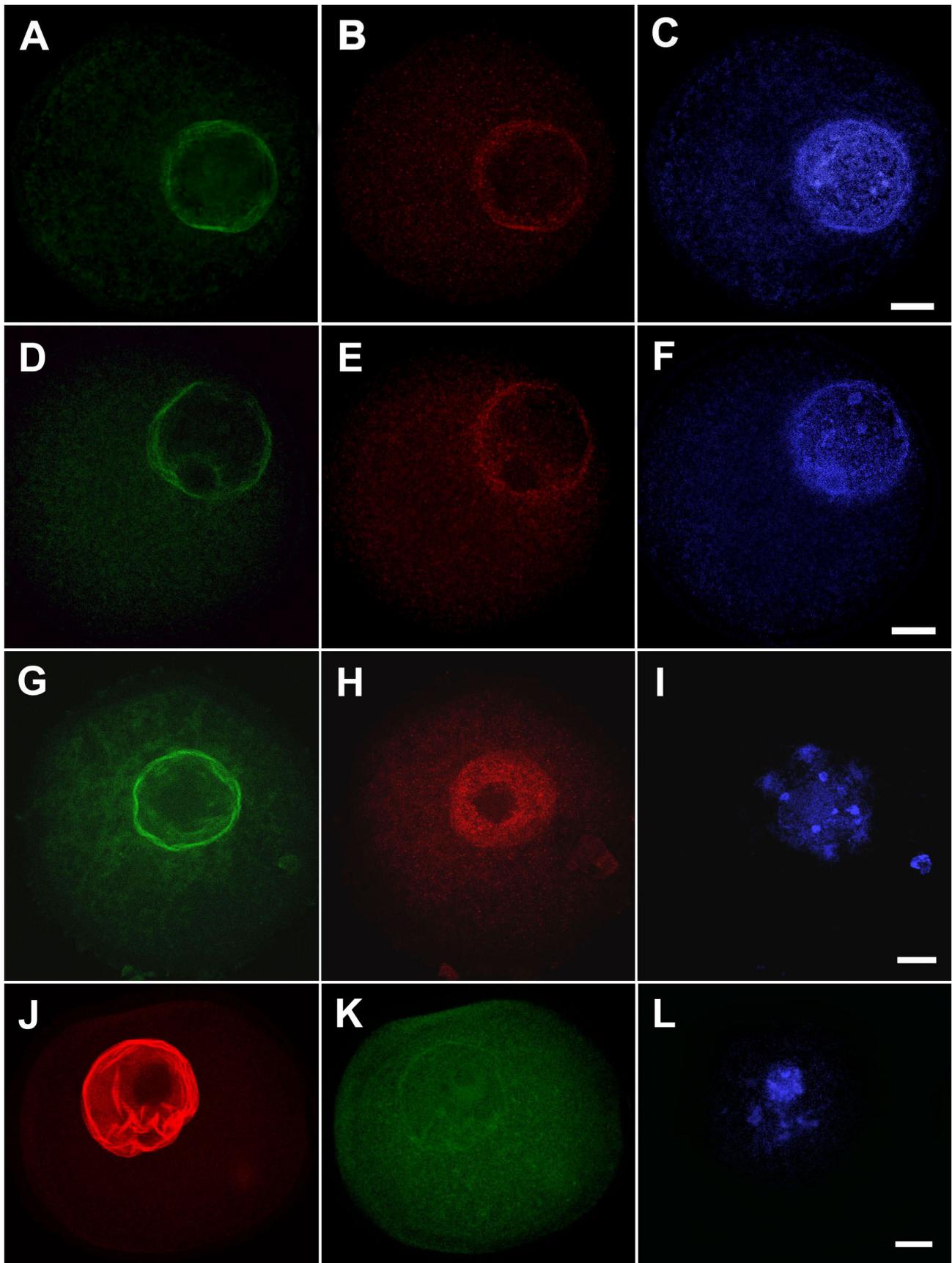


Fig. 1. Oocytes in GV stage, confocal microscopy. **A-C.** Early GV stage. **D-F.** GV stage with karyosphere. **G-I.** Late GV stage. **J-L.** Late GV / transition to GVBD. **A, D, G.** Reaction for lamin A/C. **J.** Reaction for B lamin. **B, E, H.** Reaction for Nup160. **K.** Reaction for α -tubulin. **C, F, I, L.** Reaction for chromatin in the same cells. Bars, 10 μ m.

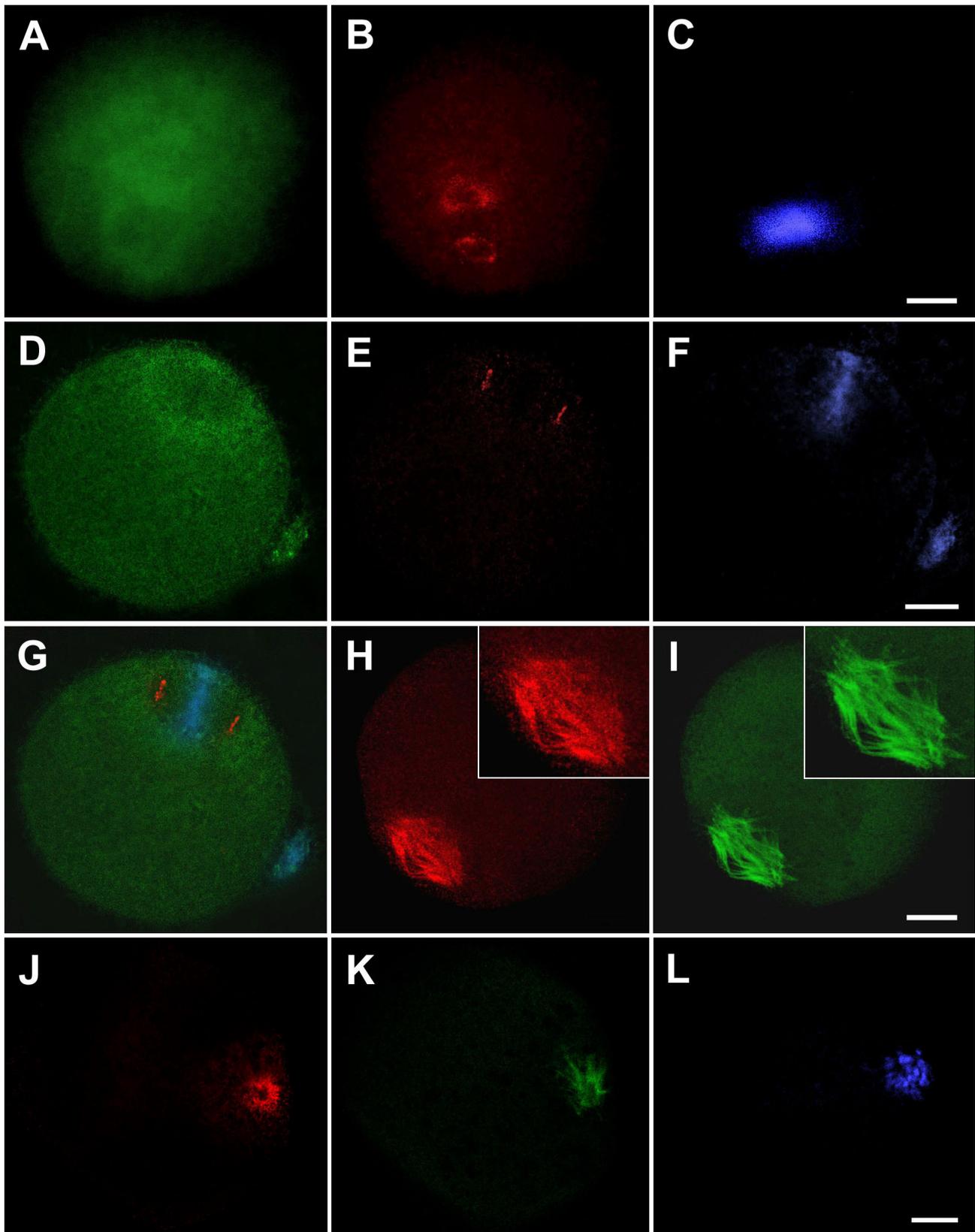


Fig. 2. Metaphase oocytes. **A-C.** Metaphase I, epifluorescence. **D-G.** Metaphase II, confocal microscopy. The polar body is seen at bottom right. **G** is a combined image of **D-F**. **H-I.** Metaphase I, confocal microscopy; insets show the spindle magnified. **J-L.** Metaphase I, confocal microscopy; the optic section includes half of the spindle with the metaphase plate and one of the poles. **A, D.** Reaction for lamin A/C. **B, E, J.** Reaction for Nup160. **H.** Reaction for lamin B. **I, K.** Reaction for α -tubulin. **C, F, L.** Reaction for chromatin. Bars, 20 μ m.

of both DNA and envelope proteins from its periphery, in a temporally coordinated manner.

It should be noted that the removal of nuclear pore complexes from the nuclear envelope indicated by the inward relocation of Nup160 was not accompanied by abolition of nucleo-cytoplasmic transport, but on the contrary, by penetration of cytoplasmic components into the nucleus as shown by the reaction for tubulin. In a recent study, we have detected other cytoplasmic proteins, namely cytokeratins and vimentin, in the nucleus of oocytes at this maturation stage (Markova et al., 2015). These observations could be explained by the appearance of large openings (10–20 times the size of nuclear pores) in the nuclear envelope of late GV oocytes described by Choi et al. (1996). While specific recruitment of cytoplasmic proteins to the nucleus is possible, it seems more likely that the size of these openings allows free exchange of macromolecules between the GV and the cytoplasm, although the nuclear envelope still exists as a microscopic object.

While the above-described details in lamin localization are new, in general our results concerning GV oocytes are in accordance with the published data. In metaphase oocytes, however, our detection of both types of lamins contrasts with earlier studies describing their disappearance after GV stage (Schatten et al., 1985; Prather et al., 1989; Prentice-Biensch et al., 2012). According to these reports, lamins are undetectable in metaphase I and metaphase II oocytes and reappear only in the pronuclear zygote. However, if nuclear lamins truly disappear from maturing oocytes, this would require a mechanism for their specific degradation at GVBD stage followed by rapid and massive *de novo* synthesis in the zygote. Moreover, a study using protein synthesis inhibitors on early bovine embryos indicates that lamin A/C in the zygote is maternal in origin rather than newly synthesized (Kelly et al., 2010). In this respect, lamin preservation and redistribution during oogenesis seems more plausible than their degradation. The negative reaction reported in earlier works may have been due to methodological difficulties associated with specific localization of protein components in metaphase oocytes. In this respect, anchoring of lamin B to membrane vesicles makes its detection particularly challenging. This may explain why the lamin B spindle matrix in somatic cells was described only a decade ago (Tsai et al., 2006) and why newer studies on oocyte lamins tend to report more details on the dynamics of lamin A/C (Foster et al., 2007) or to be entirely restricted to this lamin (Prentice-Biensch et al., 2012; Susor et al., 2015) despite the more fundamental role of lamin B. The granular pattern of lamin B localization in our metaphase oocytes could be explained by its association with membrane vesicles, products of the nuclear envelope breakdown.

It is noteworthy that, while all three studied proteins co-localized with condensed chromatin in the nuclear periphery at GV stage, and two of them (lamin A/C and Nup160) also associated with the karyosphere hetero-

chromatin, no association with the metaphase chromosomes was observed. It could be hypothesized that the interaction of these nuclear proteins with GV chromatin is mediated by some chromatin component which dissociates from chromosomes or becomes inaccessible at advanced stages of their condensation.

The present data suggest redistribution of lamins in maturing mouse oocytes similar to that described for mitosis of somatic cells. At metaphase I and II, lamin B co-localized with tubulin, forming a “spindle matrix”. Lamin A/C showed diffuse reaction throughout the cytoplasm similarly as in somatic cells. Unlike the somatic cells, however, oocytes also displayed a lamin A/C-rich diffuse halo surrounding the spindle. While it could be due to some cell type-specific localization mechanism, we would suggest passive accumulation of lamin A/C in this organelle-free area as a simpler and more plausible explanation. The brightly stained surroundings of the metaphase I plate presumably corresponded to the lamin A/C perichromosomal reaction described in metaphase I mouse oocytes by Susor et al. (2015). However, unlike these authors, we observed the same reaction pattern in mature metaphase II oocytes.

Unlike lamins B and A/C, nucleoporin Nup160 in the studied oocytes showed a distribution pattern markedly different from that in dividing somatic cells. During mitotic prometaphase, it co-localizes with the poles of the forming spindle, then spreads along the fibres in (+) direction and, at the transition to metaphase, entirely dissociates from the spindle (Orjalo et al., 2006; Güttinger et al., 2009). In the present study, however, the reaction for Nup160 in spindle poles confirmed by co-localization with tubulin persisted even in mature metaphase II-arrested oocytes. In other words, oocyte meiotic metaphase I and metaphase II resembled early prometaphase rather than metaphase of mitotic cells with regard to the Nup160 intracellular localization.

In conclusion, the present study detected nuclear lamins B and A/C and nucleoporin Nup160 at different stages from GV to metaphase II and for the first time demonstrated changes in the localization of these nuclear proteins during mammalian oocyte meiotic maturation. The distribution patterns of both lamins closely paralleled the respective stages of mitosis, while Nup160 in metaphase oocytes localized to spindle poles as described for mitotic prometaphase rather than metaphase. Taking into account the role of Nup107-160 in mitotic spindle assembly, it can be hypothesized that its peculiar distribution observed in this study reflects important meiosis-specific characteristics of spindle organization and its regulation in oocytes.

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