# DYNAMICS OF NUCLEAR ENVELOPE AND KARYOSPHERE IN LATE PHOPHASE I OF OOCYTE MEIOSIS

**Irina Chakarova, Stefka Delimitreva, Venera Nikolova, Ralitsa Zhivkova, Valentina Hadzhinesheva, Maya Markova**

*Department of Biology, Medical Faculty, Medical University of Sofia, BG-1431 Sofia, Bulgaria*

*E-mail: irra_20@abv.bg*

## ABSTRACT

Late meiotic prophase I is crucial for oocyte maturation. Cells at this stage still possess a nucleus, traditionally called germinal vesicle. Inside it, a circular layer of condensed chromatin surrounds the inactivated nucleolus. This structure is known as karyosphere. The transition between prophase and metaphase I includes major chromatin rearrangement and gradual nuclear envelope breakdown. Our aim was to correlate oocyte karyosphere status with its nuclear envelope changes indicated by altered localization of structural proteins. For this purpose, lamins, nucleoporin 160, actin and alpha-tubulin were detected immunocytochemically, actin was stained with phalloidin and chromatin with Hoechst 33258. We observed the following processes: Initially, lamins and nucleoporin were localized in the nuclear periphery while actin and tubulin were restricted to the cytoplasm. With the formation of karyosphere, part of lamin A/C and nucleoporin immunoreactivity was transferred to it, forming two concentric spheres. In oocytes with advanced chromatin condensation, the inner sphere showed a brighter reaction, nuclear envelope was visibly deformed, and actin and tubulin were detected in the nucleus in addition to cytoplasm. These results indicate a temporal correlation between karyosphere dynamics and the increasing permeability of oocyte nuclear envelope.

***Key words:*** *oocyte meiosis, karyosphere, nucleoporin 160, nuclear envelope*

## INTRODUCTION

Female mammals are born equipped with a pool of immature oocytes in their ovaries. These oocytes are arrested in prophase of the first meiotic division and will be gradually used during reproductive age. With each estrous or menstrual cycle, a few of the stored cells are “awakened” from their dormant state and undergo a differentiation process called oocyte maturation. It includes meiotic stages from late prophase I to metaphase II, when the cell is ovulated and ready to be fertilized. The initial stage of oocyte maturation, i.e. late prophase I, is crucial for the correct course and successful completion of later stages. It is also known as germinal vesicle stage, because during it the oocyte still possesses a nucleus, traditionally called germinal vesicle [Li & Albertini, 2013].

When the immature oocyte is activated and resumes meiosis, the microscopic appearance of its nucleus changes as chromatin undergoes major remodeling. The nucleolus stops transcription, becomes spherical and is encircled by condensed chromatin. As a result, a dense circular perinucleolar rim called “surrounded nucleolus” or karyosphere is formed [De La Fuente et al. 2004]. This structure, observed not only in mammals but also in many other animal groups, assembles all chromosomes together in a limited nuclear volume [Gruzova & Parfenov 1993]. As the oocyte progresses through germinal vesicle stage, chromosomes detach from the nuclear envelope and undergo condensation. The karyosphere is finally lost at the transition to metaphase I.

While oocyte chromatin undergoes remodeling, nuclear envelope is also changed. Its permeability gradually increases and its stability decreases [Choi et al. 1996]. Finally, it breaks down into a number of small membranous vesicles. Chromosomes are now free in the cytoplasm and a meiotic spindle forms around them. This short transitional stage is called germinal vesicle breakdown. It is soon followed by metaphase I.

Karyosphere formation is thought to facilitate transcriptional silencing of oocyte chromosomes and their subsequent arrangement in the metaphase I plate. Experimental studies have

shown a correlation between karyosphere presence and the ability of the oocyte to resume meiosis, to reach metaphase II and, if fertilized, to develop into a multicellular embryo [Lodde et al. 2007]. These data and the phylogenetic conservation of karyosphere suggest its structural significance. However, its dynamics has not yet been temporally correlated to other processes in the maturing oocyte and in particular to nuclear envelope changes. Our previous research has shown that nuclear envelope proteins lamin B, lamin A/C and nucleoporin 160 as well as cytoskeletal proteins alpha-tubulin and actin are present in the germinal vesicle-stage oocyte and undergo redistribution during meiotic maturation [Nikolova et al., 2015, 2017]. The present study aimed to correlate oocyte karyosphere status with its nuclear envelope changes indicated by altered localization of these structural proteins.

## MATERIALS AND METHODS

Prepubertal virgin mice of strain BALB/c were used as oocyte source in accordance with European Union and Bulgarian legislature and after approval by the institutional Ethics Commission. Oocytes at various maturation stages were obtained and processed as described before [Nikolova et al., 2017]. Briefly, the animals were hormonally stimulated by 5 IU Menogon (Ferring, Kiel, Germany) and euthanized 40 h later. Oocytes were retrieved from ovaries, treated with 0.5 mg/ml hyaluronidase (Sigma-Aldrich) to remove cumulus oophorus and fixed in 2% paraformaldehyde and 0.04% TritonX-100. Lamin B, lamin A/C, nucleoporin 160 and alpha-tubulin were detected by indirect immunofluorescence using specific primary and secondary antibodies. Actin was stained with labeled phalloidin and chromatin with the fluorescent dye Hoechst 33258. All listed reagents for specific detection and visualization were products of Sigma-Aldrich.

## RESULTS AND DISCUSSION

According to their karyosphere status, oocytes were classified into three groups: (1) with no visible karyosphere, thought to be in early germinal vesicle stage; (2) with formed karyosphere, indicating resumed meiosis and ability to progress through maturation; (3) with prominent karyosphere and condensing chromosomes, presumed to be at the end of germinal vesicle stage and preparing for germinal vesicle breakdown and transition to metaphase I. The three steps of chromatin rearrangement during karyosphere formation is illustrated in figure 1.

In group 1 oocytes, all three studied nuclear envelope proteins (lamins B and A/C and nucleoporin 160) were localized in the nuclear periphery while cytoskeletal proteins actin and tubulin were restricted to the cytoplasm. This distribution of structural components to their traditional domains was in accordance with earlier reports (e.g. [Sanfins et al. 2004]). It was interpreted as characteristic for dormant, prophase-arrested oocytes and possibly for oocytes in which maturation had just started and had not yet influenced the visible structure of the cell, i.e. early germinal vesicle stage.

With the formation of karyosphere (group 2 oocytes), part of lamin A/C and nucleoporin immunoreactivity was transferred to it, forming two concentric spheres. This redistribution could be explained as reflecting, on one hand, the role of the karyosphere in prophase I chromatin remodeling, and on the other hand, a degree of structural relaxation of nuclear envelope and disassembly of nuclear pore complexes. However, actin and tubulin remained confined to the cytoplasm, showing that nuclear envelope was still functional as an effective barrier between the nuclear interior and the cytosol and had not yet changed its permeability for macromolecules.

In group 3 oocytes presumed to be in late germinal vesicle stage, the prominent karyosphere showed a brighter reaction for lamin A/C and nucleoporin 160 than the nuclear envelope. The latter was still labeled by the anti-lamin B antibody but was visibly deformed, indicating advanced

destabilization and imminent breakdown. Another important finding at this stage was the presence of actin and alpha-tubulin immunoreactivity in the nucleus in addition to cytoplasm, a sign that the nuclear envelope was no longer performing its barrier function. In other words, despite the disassembly of nuclear pore complexes which released their key component nucleoporin 160, nucleo-cytoplasmic transport was not abolished. On the contrary, it became uncontrolled, allowing cytoplasmic proteins without any nuclear localization signal to enter the nucleus. Choi et al. [1996] have reported the presence of openings much larger than nuclear pores in the germinal vesicle envelope shortly before its breakdown. We suppose that such openings were present in our group 3 oocytes and were responsible for the observed free access of actin and tubulin to the nucleus. In conclusion, the results of the present study show a temporal correlation between karyosphere dynamics and the increasing permeability of nuclear envelope during late prophase I of oocyte meiosis.

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Legend for Figure 1.
The maturing oocyte is surrounded by zona pellucida. **A-** In the germinal vesicle (GV), functional nucleolus still has its usual appearance. **B-** The nucleolus is visibly disaggregated. **C-** A nearly central GV region is surrounded by heterochromatin. **D-** This region is tightly bordered by a heterochromatin sphere.