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Review Articles

Kariosphere, the Enigmatic "Surrounded Nucleolus" of Maturing Oocytes: Review

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In mammals and other animals, the late prophase I oocyte undergoes large-scale chromatin remodeling. Condensing chromosomes associate with the inactive nucleolus and surround it with a rim of heterochromatin called karyosphere. This rim has been shown to contain centromeric and pericentromeric regions of chromosomes. Karyosphere formation coincides in time with global transcriptional silencing of oocyte genes, but this seems to reflect regulation by common upstream factors rather than causal relationship between the two processes. The function of karyosphere is not yet known, but is likely related to the positioning of bivalents in metaphase I by clustering chromosomes together in a limited volume before the nuclear envelope breakdown. Studies show that karyosphere formation, ("non-surrounded to surrounded nucleolus transition") indicates acquisition of meiotic and developmental competence by the oocyte. Methodological approaches are discussed to use this important morphological marker to select oocytes with better potential for assisted reproduction.

Key words: oocytes, meiosis, chromatin rearrangement, heterochromatin

The karyosphere as a temporary structure of germinal vesicle stage oocytes

In mammals, as in most other vertebrates and a number of invertebrate phyla, the mature oocyte at the time of fertilization is arrested in meiotic metaphase II and, hence, has no nucleus. In the immature oocyte, however, the nucleus plays a key role during the long prophase I arrest and the early stages of meiotic maturation. This nucleus, also known as germinal vesicle (GV), is unusual and remarkable in many respects. Its chro-

matin undergoes major rearrangements called by some authors "large-scale" changes or remodeling [11]. These changes transform the initially typical nucleolus of the oocyte into a transcriptionally inactive "nucleolus-like body" [10, 17]. In the final stage of meiotic prophase I, heterochromatin accumulates in a dense circular perinucleolar rim, forming a structure called surrounded nucleolus, rimmed nucleolus or karyosphere [5]. To be distinguished from it, the nucleolus of the early primary oocyte, which has a typical structure and function, is called non-surrounded nucleolus. The karyosphere is a result of all oocyte chromosomes joining in a limited nuclear volume with final formation of a single complex chromatin structure. It has been described in members of distantly related animal groups such as mammals, birds, amphibians, insects and annelids, suggesting a phylogenetically conserved process in metazoan oogenesis [7]. The scope of the present review is limited to mammals as a group relevant for the understanding of human oogenesis.

After a period of proliferation by mitosis, mammalian female germ cells in the fetal ovary undergo a transformation from oogonia to primary oocytes and start meiosis. They complete the first stages of prophase I, including the meiotic recombination (crossing over), and upon reaching diplotene are arrested for weeks, months or years, depending on the species. During this prolonged arrested diplotene, often referred to as dictyate stage, the prophase chromosomes are decondensed to a loose chromatin mass [16]. The reproductive period of the female, beginning at puberty, is characterized by secretion of hormonal signals that stimulate follicle growth and bring oocytes out of their quiescent state. With each menstrual/estrus cycle, a cohort of oocytes resumes meiosis. One or more of them will complete the 1st meiotic division, extrude the 1st polar body, start the 2nd meiotic division and reach its metaphase, where meiosis is arrested again until fertilization. These stages from late prophase I to metaphase II are collectively called "oocyte meiotic maturation" [12]. We shall discuss only its initial step, the "germinal vesicle" stage when the primary oocyte still possesses an intact nucleus until its envelope is disassembled at the "GV breakdown" stage, marking the transition to metaphase I.

Morphological classification of oocyte nucleoli

Oocytes isolated from growing preantral and antral follicles can be classified into discrete patterns according to the morphological organization of the chromatin in their nuclei. Initially, oocyte chromatin is mostly decondensed and dispersed throughout the nuclear volume. Then, the degree of condensation increases, and condensed chromatin foci start to associate with the nucleolar periphery. This continues until the nucleolus is encircled by a complete rim of heterochromatin, forming the karyosphere or "surrounded nucleolus" (**Fig. 1**). The described structural remodeling coincides with decrease and cessation of transcriptional activity, both in the nucleolus and outside it. These changes have been described in the human [16], mouse [14] and a number of other mammalian species. The morphological configuration in which the perinucleolar chromatin rim is absent is named "non-surrounded nucleolus" (NSN), while the configuration with present perinucleolar chromatin rim (karyosphere) is termed "surrounded nucleolus" (SN). Some authors also describe the intermediate configurations as "partly NSN" and "partly SN". The process shows some species specificity, as shown by horse and bovine oocytes. In them, chromatin condenses into a single compact clump instead of forming a clear rim around the nucleolus. For that reason, cattle prophase oocytes are classified into four stages designated, respectively, as GV0 (diffuse pattern of chromatin), GV1 (few foci of condensation), GV2 (distinct clumps of condensed chromatin)

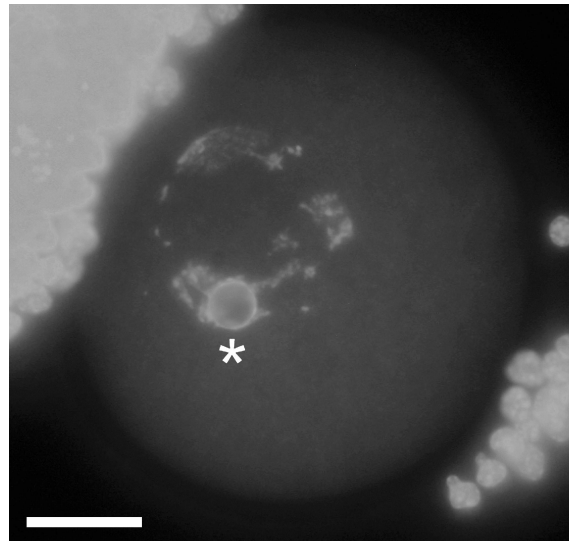

Fig. 1. Mouse oocyte in late GV stage, surrounded by follicle cells, stained with the chromatin dye Hoechst 33258. The karyosphere, indicated by an asterisk, is visible as a circular structure inside the nucleus. Several condensing bivalents are seen attached to it. Bar = 20 μm. Reprinted from [12]

and GV3 (chromatin organized into a single clump). Nevertheless, detailed microscopic studies have revealed that chromatin in GV3 oocytes is located near the inactive nucleolus, resembling the SN configuration of mouse and human [9]. This is in accordance with the theory that karyosphere formation is a phylogenetically conserved process across the mammalian class and even beyond it.

Composition of the karyosphere and the inactive nucleolus inside it

With regard to the reported presence of a karyosphere in the late GV-stage oocytes of many animal species, the question arises about the composition of the enclosed inactive nucleolus (or "nucleolus-like body") and the associated chromatin regions forming the karyosphere. Cytochemical and immunocytochemical studies reveal that the interior of the "surrounded nucleolus" is rich at proteins, including typical nucleolar proteins involved in rRNA processing and ribosome biogenesis. It is positive also for RNA, but nature of this RNA is still a mystery, because *in situ* hybridization experiments with specific probes show low rRNA content [17]. The composition and rearrangement of condensed chromatin regions forming the karyosphere has been studied by fluorescent *in situ* hybridization. In early GV-stage oocytes before karyosphere formation (NSN configuration), probes for centromeric and pericentromeric DNA sequences label masses of chromatin (chromocenters) in different regions of the nucleus. These chromocenters often have more than two centromeric spots, indicating clustering of two or more chromosomes. In late GV oocytes with karyosphere, centromeric and pericentromeric sequences are located in its rim. Curiously, while the general level of chromatin condensation increases, pericentromeric heterochromatin seems to undergo partial de-

condensation from the NSN chromocenters to the SN perinucleolar rim, allowing it to spread around the nucleolus and encircle it completely. Meanwhile, nucleolar organizer region become condensed during the NSN to SN transition and move out of the nucleolus proper to join the karyosphere heterochromatin, a rearrangement which reflects their transcriptional silencing [4]. It seems that the layer of heterochromatin forming the karyosphere effectively isolates its interior: according to our observations, in late GV oocytes, nuclear envelope is no longer a barrier for cytoplasmic proteins and they can be detected inside the nucleus, but not inside the karyosphere [13].

Relationship between karyosphere formation and transcriptional silencing

The temporal correlation between karyosphere formation and cessation of transcription in the oocyte nucleus has been mentioned early by microscopic observations of oocytes allowed to incorporate labeled nucleotides [16]. In the mouse, NSN oocytes are transcriptionally active and synthesize all classes of RNA, while SN oocytes are characterized by global repression of transcription [10]. This raises the question whether the condensation of chromatin leading to karyosphere formation is a mechanism of transcriptional repression. However, detailed studies have shown that large-scale chromatin changes and global transcriptional silencing can be experimentally dissociated. For instance, in oocytes from nucleoplasmin-2 knockout mice, transcription is stopped even though karyosphere fails to form [6]. On the other hand, in oocytes from histone 3-lysine 4 methyltransferase (MLL2) conditional knockouts, transcription is not silenced even though karyosphere formation is successful [1]. These data suggest that there is unlikely to be a direct causal relationship between large-scale chromatin remodeling and transcriptional silencing; rather, these two processes might normally be controlled by common upstream factors, which are unfortunately still unknown.

Epigenetic events correlating with karyosphere formation

Attempts have been made to link the transcriptional silencing of late GV oocytes to epigenetic markers. In bovine oocytes, the down-regulation of transcription that accompanies even the early steps of chromatin condensation correlates with a substantial increase in global DNA methylation [10]. On the other hand, acetylated histones known to keep the chromatin in a transcriptionally permissive state do not disappear during the NSN to SN transition but are found associated with the condensing chromatin, including the karyosphere [19]. A study including immunolocalization of several modified histones associated with either activated or repressed state has found these epigenetic markers to follow chromatin movements during the NSN to SN transition and to aggregate in the karyosphere without distinction between active and repressive signals. The authors conclude that the germinal vesicle may have a specific histone modification landscape and the common rules of the histone epigenetic code known from somatic cells are not valid for the oocyte [4].

If karyosphere formation is not causally related to transcriptional silencing and shows no straightforward correlation with the epigenetic reprogramming of the oocyte genome, its function could be related to the next step of oocyte maturation. The germinal vesicle, even in the small eutherian oocytes, is so large that the subsequent assembly of all bivalents in a metaphase plate becomes problematic. It has been hypothesized that microfilaments, which in metaphase I organize around the meiotic spindle into a spin-

dle-like structure, keep the bivalents together in a limited volume [2]. However, while this process could play an auxiliary role, it is not indispensable for the alignment of bivalents in a metaphase plate, because disruption of microfilaments by cytochalasin does not prevent the formation of a normal metaphase I spindle [8]. Karyosphere formation, which includes condensation and clustering of oocyte chromosomes in a limited nuclear volume, is expected to facilitate their subsequent positioning in a metaphase plate and to reduce the chance of isolated bivalents remaining outside the meiotic spindle. Data in accordance with this view are provided by time-lapse imaging of chromatin-stained mouse oocytes, which shows a 12% reduction in the germinal vesicle area immediately before its breakdown [3].

Correlation of oocyte meiotic and developmental competence with karyosphere formation

Perhaps the most significant finding about the karyosphere of GV-stage mammalian oocytes is the correlation between its presence and the ability of the oocyte to resume meiosis, to reach metaphase II and, if fertilized, to develop beyond the first mitotic division of the zygote. In 2002, a study on oocytes isolated from mouse antral follicles showed that most cells without karyosphere (the NSN configulation) failed to resume meiosis. Only 15% of oocytes with this chromatin configuration reached metaphase II and, upon fertilization, none of the obtained zygotes progressed beyond the 2-cell stage. By contrast, 75% of oocytes with karyosphere (the SN configuration) matured to metaphase II and, when fertilized, 47% of them developed to 4-cell stage and 18% to blastocyst [20]. Later studies, using mouse oocytes as well as oocytes from other mammals, confirmed that meiotic competence (i.e. the ability of the oocyte to resume meiosis and to reach metaphase II) is acquired at the time when chromatin condensation starts, while developmental competence (i.e. the ability of the oocyte, if successfully matured and fertilized, to develop beyond the 2-cell stage) is acquired slightly before the chromatin reaches its highest level of condensation [9]. This striking difference in oocyte potential is most likely due to accumulation of important regulatory proteins and mRNAs for them during the NSN-to-SN transition, notably the transcription factor OCT4, which is a marker of pluripotency [19].

The reliability of the karyosphere as an indicator of the oocyte developmental potential has prompted some researchers to seek a method to select oocytes with karyosphere for use in assisted reproduction. There are already reports of such protocols in which oocytes are sorted according to their chromatin configuration, revealed by supravital staining with Hoechst 33342 [15]. Unfortunately, intercalating dyes greatly increase the incidence of frameshift mutations, which makes this approach unsuitable for use in assisted reproduction of valuable animals and especially of humans. Selection of oocytes with high developmental potential based on the presence of karyosphere may become possible with the eventual introduction of other, less invasive methods to assess the chromatin configuration.

Conclusions

Although the precise functions of the karyosphere and the molecular mechanisms of its formation remain to be elucidated, it should be regarded as one of the most important cell type-specific structures of the maturing oocyte. This is evident from the correlation between its presence and the acquisition of meiotic and developmental competence, as

well as its phylogenetic conservation in the animal kingdom. In assisted reproduction, where the quality of oocytes is of crucial importance, the use of the karyosphere as a marker of meiotic and developmental competence to select cells with better potential could improve the outcome. However, this requires development of new methodological approaches for non-invasive assessment of the oocyte chromatin configuration.

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