Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta Morphologica et Anthropologica, 29 (3-4)
Sofia ● 2022

The Odd Behavior of the Nuclei in Maturing Mammalian Oocytes and Zygotes

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Meiosis in the ovary has some important differences both from mitosis and from meiosis in the testis. The main reason for this is the extremely large cytoplasm of the oocyte. Meiotic maturation is related to global chromatin rearmament. In that huge volume, the chromatin division requires cooperative support by nuclear and cytoplasmic factors. That is why, the oocyte nucleus (known as germinal vesicle), is unusual in many respects: the nuclear envelop in oocytes is not a real border between the nucleus and cytoplasm; the nucleolus does not function as real nucleolus; the centrioles are destroyed during prophase and meiotic spindle is constructed by smaller microtubule organizers; the chromosomes possess autonomy; the pronuclei in the zygote and the nuclei in the very first blastomeres also have unusual behavior – the multiple nucleoli in the male and female pronuclei are not real nucleoli; paternal and maternal genomes possess their own territory and autonomy.

Key words: meiosis, oocyte, zygote, karyosphere, germinal vesicle

In most vertebrates and in a number of invertebrates, the mature oocyte is arrested at the metaphase stage of the second meiotic division. Hence, at that moment the oocyte has no nucleus but a meiotic spindle. The onset of anaphase is not triggered, and meiosis does not finish if the contact with a spermatozoon did not occur. The organization of the genetic material in mature oocytes is really odd and that oddness has its roots in the oogenesis.

Traditionally, the oocyte nucleus is not called nucleus. The maturing oocytes are arrested initially at prophase I. In mammals, this sleeping period can be several years long, or even decades. The meiosis awakes after hormonal signals and continues to the metaphase II arrest in the oocytes that are ready for ovulation and fertilization. During the long prophase I arrest, waking, and till the metaphase II arrest, the oocyte chromatin is organized in an unusual nucleus. This nucleus is also known as germinal vesicle (GV). This traditional name was introduced by Jan Evangelista Purkyně in 1825, who used it for the nucleus of the hen egg. Several years later, his doctoral student

A. Bernhardt used “germinal vesicle” as a name for the nucleus in mammalian oocyte. Somehow, the name “germinal vesicle” survived and even now the embryologists use that name instead of “nucleus”. Perhaps the reason to keep the strange old name is that the researchers always intuitively knew that this structure is not an ordinary cell nucleus. Now we know – it was a good decision.

What is the karyosphere. During the total chromatin rearrangement, the typical nucleolus is transformed into a transcriptionally inactive sphere (nucleolus-like body). In the end of meiotic prophase I, a rim of heterochromatin is accumulated around the original nucleolus. The result is an absolutely spherical structure. In different papers it is referred to as “surrounded nucleolus”, “rimmed nucleolus” or “karyosphere”. Its border is made of the most compact parts of the chromosomes. Hence, at that moment all chromosomes are located in a limited nuclear volume around the karyosphere. The construction of the karyosphere starts with relocation of several centromeric and pericentromeric regions towards the nucleolus [5]. The process continues till the creation of full and bold heterochromatin rim. Curiously, at that time the general level of chromatin condensation increases, but the pericentromeric regions undergo partial decondensation. That makes them able to spread around the nucleolus. Together with this, the nucleolar organizer regions, that normally are in the nucleolus, move out of it and become condensed.

The karyosphere is a protected room. It seems that the heterochromatin border around the karyosphere very effectively isolates its interior. According to our observations [3], at late GV sates the nuclear envelope is not an effective barrier between the nucleus and the cytoplasm – cytoplasmic proteins can enter the nucleus, but they are not detected inside the karyosphere. The interior of the karyosphere contains proteins which are involved in the typical nucleolar functions – rRNA processing and ribosome construction. It contains also small amount of RNA, but the nature of this RNA is still not clear.

The karyosphere keeps the chromosomes in a limited volume. The construction of the karyosphere keeps the chromosomes clustered in a limited volume. That facilitates their transfer and positioning in the meiotic spindle. Also, the clustering reduces the risk to have chromosomal bivalents outside the spindle. The microfilaments are involved in the chromosome clustering that keep the bivalents together in a limited volume. That is in accordance with the fact that at metaphase I, the microfilaments organize an additional spindle-like structure around the tubulin meiotic spindle [7].

The nuclear envelop in oocytes is not a border between the nucleus and cytoplasm. The volume of GV is dramatically reduced after the appearance of the karyosphere, immediately before the germinal vesicle breakdown. That is the moment to start construction of the meiotic spindle. This process totally changes the nuclear architecture. During these steps of oocyte maturation, the behavior of cytoskeletal fibers is very specific. That is related to the following facts: the oocytes are extremely large cells, even in mammals (human oocyte diameter is 100-120 μm); the nucleus occupies very large territory (up to 40 μm); several hours before the nuclear envelope breakdown, nuclear pores lose their function to be checkpoints for the transport through the nuclear envelope; large openings (up to 800 nm in diameter) appear in the envelope; their function is to provide access of cytoplasmic proteins, including cytoskeletal elements, to the chromatin.

Our investigations revealed that at this moment the shape of the nucleus remains visibly normal, but in fact it stops being a separate compartment of the cell. When the karyosphere is constructed, the nuclear envelope gradually loses its integrity; cytoplasmic cytoskeletal elements enter the nucleus; GV stage is transformed into GVBD stage (germinal vesicle breakdown). During this transformation, the nucleus becomes positive for alpha-tubulin and fibrillar actin [4]. Only the interior of the karyosphere stays free of actin and tubulin. Outside the karyosphere, the most intensive reaction for actin and tubulin we detected at the places with the most condensed chromatin.

There are no centrioles in the oocyte. The fact that the maturing oocytes lose their centrosomes makes the probability to start development without egg-sperm fusion nearly zero. The oocyte centrioles are destroyed at prophase I. The chromatin takes the responsibility to arrange the spindle by itself – the chromosomes serve as platforms for creation of multiple microtubule asters that later fuse to construct a bipolar spindle. An additional problem here is the distance. The microtubules are unable to bind effectively chromosomes if the cells diameter exceeds 30 μm. In the oocytes, the chromosome relocation is mediated by the other types of cytoskeletal fibers – microfilaments and intermediate filaments. That is why, they must enter the GV.

Every chromosome is able to create its own spindle-like structure and its own nuclear envelope. The fact that the chromosomes are responsible to organize the microtubules has some unusual consequences. First, a small group of chromosomes, even single chromosomes separated from the main chromosomal set, are able to arrange microtubules into a small spindle-like structure around themselves. Second, the sperm chromosomes can use that autonomy to create a spindle for themselves. In this case, in the oocyte cytoplasm together with the oocyte spindle that is arrested at metaphase II, another spindle can be observed – a spindle with a sperm tail [2]. Third, the autonomy of the chromosomes is used to facilitate integration-disintegration cycle of the nuclei during the first divisions of the huge zygote – for example: in fish and amphibian embryos, single chromosomes are surrounded by their own nuclear envelopes; these structures are referred to as karyomeres; later the karyomeres fuse to form a common nucleus [1].

Paternal and maternal genomes possess autonomy. It was previously thought that a single microtubule spindle is responsible to combine the male and female genomes and then to create a two-cell embryo. In fact, in the mouse zygote two bipolar spindles are formed. They look like a single structure, but the maternal and paternal genomes are located in two independently arranged parts which must align to each other perfectly before the anaphase onset [6]. If the alignment fails, after the first zygote division blastomeres with more than one nucleus will be created. The two halves of the first mitotic spindle in the zygote hold the two parental genomes separated, so the maternal and paternal chromosomes do not mix. They do that in the subsequent developmental stages. This mechanism of dual-spindle assembly is a probable explanation for the relatively high level of morphological and chromosomal abnormalities in human embryos observed in the fertility clinics.

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