Centriolar satellites associate with condensed chromatin in early mouse oocytes and undergo redistribution during transition to dictyate

Valentina Prodanova Hadzhinesheva, Irina Valcheva Chakarovaa, Stefka Metodieva Delimitrevaa, Maya Dyankova Markova, Venera Pantaleeva Nikolova, Milena Sergeeva Mourdjevab, Pavel Istiliyanov Rashevc and Ralitsa Stefanova Zhivkova

1Department of Biology, Medical Faculty, Medical University of Sofia, Sofia, Bulgaria; 2Department of Molecular Immunology, Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria; 3Department of Immunobiology of Reproduction, Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria

ABSTRACT
In eukaryotic cells during interphase, the centrosome is positioned close to the nucleus by a yet unidentified mechanism and determines the basic cell polarity. The aim of this study was to trace the dynamics of centriolar satellite protein PCM-1 in newborn mouse ovaria. Ovarian sections of newborn mice were subjected to immunofluorescent analysis. The intracellular localization of PCM-1 in prophase I oocytes was compared between 1- and 2-day-old mice. In oocytes from 1-day-old ovaria, double or broader single discrete PCM-1 aggregates with perinuclear localization were observed in association with condensed chromatin regions. In oocytes from 2-day-old ovaria, PCM-1 structures were less discrete and chromatin was homogenous. In some oocytes, single or double PCM-1 aggregate was still recognizable, while in others, the PCM-1-containing structure was crescent-shaped. Our study describes for the first time reorganization of centriolar satellite protein PCM-1 in prophase I oocytes from developing primordial follicles correlated with chromatin changes during transition to dictyate.

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Introduction
In most eukaryotic cells, the centrosome acts as the main microtubule-organizing center. During interphase, it is positioned close to the nucleus by a yet unidentified mechanism and determines the basic cell polarity. During cell division, it organizes spindle assembly [1]. The centrosome is made up of a pair of centrioles and surrounding pericentriolar material. The latter is an assembly of centrosomal proteins responsible for the nucleation and organization of microtubules [2]. It includes a group of non-membrane proteinaceous particles known as centriolar satellites. In animal cells, they are small granules located in the vicinity of the centrosome [3,4].

Centriolar satellites show dynamics in their localization, associating with microtubules and moving towards their minus end. They have also been found to play a role in ciliogenesis, autophagy and proteasomal degradation [5]. The major component of centriolar satellites acting as a structural platform is the so-called PCM-1 (pericentriolar material 1) protein [6]. Inhibition or depletion of PCM-1 disrupts the radial organization of microtubules, indicating that centriolar satellites are involved in microtubule anchoring [7]. Centriolar satellites undergo reorganization during cell division. Their component PCM-1 has been found to undergo redistribution during mitosis of somatic cells, with decreased intensity and even disappearance of PCM-1 labelling of centrosomes during late G2 and throughout M phase [6,8].

In most animals, including mammals, oocyte meiosis brings reduction not only of the chromosomal number but also of the centrosome [9]. At the transition to dictyate, the centrosome is disassembled. Centrioles disappear as microscopic objects and pericentriolar material disseminates in the cytoplasm. After meiotic resumption, numerous acentriolar microtubule-organizing centres are formed from this material. They nucleate microtubules and loosely organize them, shaping the poles of the meiotic spindle [10]. This way, oocyte meiosis proceeds without centrosomes and in this respect is highly atypical for an animal cell division.
There are still many unanswered questions concerning this process due to methodological difficulties hindering its investigation. Mammalian oogenesis starts during foetal development and in most species, including human, is arrested in ditcytate before birth. The resting pool of primordial follicles containing primary oocytes that will ensure the reproductive capacity of the female is already formed in the human neonatal ovarium. In later life, the period to the start of follicle growth and maturation takes years to decades. Laboratory mice, however, are easier to study in this respect. They have a short generation time (about 10 weeks from being born to giving birth) with an average of 5—10 pups per litter and an immediate postpartum oestrus [11]. Because of this accelerated developmental timeline, mouse ovary at the moment of birth presents stages of oogenesis and folliculogenesis that in other mammals can be found only in foetuses. During the first week of newborn life, oocytes are still progressing through the first four stages of prophase I and primordial follicles are still forming [12]. This time course of oogenesis makes newborn mouse ovaria a very suitable object to study initial meiotic stages.

In early mouse oocytes entering meiosis, PCM-1 has been detected at its usual location: the vicinity of centrioles [13]. Functional studies using microinjection of anti-PCM-1 antibodies have revealed a role of this protein for the cell cycle progression of pronuclear zygotes [14]. However, to our knowledge, no published works have addressed the state of PCM-1 in oocytes after the start of meiosis and before fertilization. Due to the above-described peculiarities of mouse oocyte meiosis, reorganization of oocyte components during prophase I can be investigated by comparing the findings in newborn mouse ovaria retrieved on different days of early postnatal life. To trace the centriolar satellites, we localized their key component PCM-1 in 1- and 2-day-old mice by immunofluorescence and laser scanning confocal microscopy.

**Materials and methods**

Newborn female BALB/c mice were used: eight 1-day-old and eight 2-day-old animals. Their ovaria were isolated, formalin fixed (Leica Biosystems, Germany), paraffin embedded (Leica Biosystems, Germany) and cut into 5-μm thick slices. The sections were mounted onto adhesive microscopic slides. They were deparaffinized in xylene (3 × 10 min) and rehydrated in ethanol series (100%, 96%, 80%, 70%, 5 min in each) and distilled H₂O for 5 min just prior to the immunohistochemistry. From each group of mice, 32 ovarian slides were selected to be processed.

Immunohistochemical labelling was carried out as follows: boiling in saline-sodium citrate (SSC) buffer, pH = 6, for 20 min at 95°C and cooling at room temperature; FITC (fluorescein isothiocyanate)-protein blocking (Quartett, Germany) for 15 min; blocking of unspecific binding (Super Block, ScyTek, USA) for 5 min; incubation with first antibody against PCM-1 protein (rabbit polyclonal IgG H-262, Santa Cruz Biotechnology, USA) for 1 hour and application of the secondary anti-rabbit FITC-conjugated antibody (Sigma-Aldrich, Germany) for 1 hour. Washing in PBS (phosphate buffered saline) three times, 5 min each with 0.05% Tween 20, pH = 7.2 was performed after each of the above steps. The chromatin was stained by Hoechst 33258 (Sigma-Aldrich, Germany) for 5 min; the slides were immersed in polyvinyl-alcohol (Fluka, Germany) and the reaction was detected by confocal laser-scanning microscopy at 0.2μm optical sectioning (Leica TCS SPE, Leica, Germany).

All experiments were performed in accordance with EU and Bulgarian law and the ethical requirements of the Bulgarian Food Safety Agency for humane treatment of animals used for research.

**Results and discussion**

Due to the later time of meiotic arrest in mouse oogenesis (after birth), observations on newborn mouse ovaria allowed tracing the dynamics of selected oocyte components before dictyate. This gave the opportunity to study stages of oogenesis that in other mammalian objects would require handling of foetal ovaries. In the ovarian sections from both 1- and 2-day-old mice, developing oocytes were in the process of being surrounded by somatic cells, that is, primordial follicles were still forming. At this key stage of oogenesis and folliculogenesis, the elapsed time of 24 hours allowed important changes to take place. Therefore, it was not unexpected that the findings in oocytes differed between 1- and 2-day-old ovaria with regard to both centriolar satellite protein PCM-1 and chromatin structure.

In ovarian tissue samples from 1-day-old mice, PCM-1 was detected in oocyte cytoplasm as a single broad or double discrete aggregate adjacent to the nucleus (Figure 1A). This suggested association of this protein with some small, compact cellular structure. We suppose that the positive reaction for PCM-1 corresponds to the duplicated centrosome, the two
copies of which may remain in close vicinity or separate at some distance. Oocyte centrosomes in early mouse ovaries have a role in the organization of the Balbiani body, a transitory complex of organelles involved in oocyte differentiation across a number of animal phyla [15] and observed in mouse oocytes at a corresponding developmental stage, that is, in ovaries of 1–2-day-old mice [16]. The two PCM-1 positive structures observed by us in some oocytes could also correspond to the pair of ‘multivesicular aggregates’ concentrating the oocyte gamma-tubulin after the centrosome disassembly normally occurring in mammalian oogenesis at the transition to dictyate [9,17].

In the same ovarian sections from postnatal day 1, we observed condensed chromatin regions inside the nucleus. They presumably corresponded to meiotic chromosomes that at this stage are normally characterized by some degree of chromatin condensation. A prominent spot of condensed chromatin was always present at the nuclear periphery in the vicinity of the PCM-1 aggregate and in apparent spatial connection to it (Figure 1B). This chromatin structure could not be identified with certainty, but a parallel could be made with published reports about the association of the centrosome with the chromosomal ‘bouquet’ in zebrafish oocytes during meiotic prophase I [18].

Figure 1. Laser scanning confocal microscopy of ovarian section from a 1-day-old mouse. Two oocytes are partly surrounded by somatic cells. (A) Centriolar satellite protein PCM-1 displays discrete aggregates peripherally to the nucleus (the nuclei are marked by N). A single broad PCM-1 aggregate and a double PCM-1 structure are marked by arrows. (B) Nuclei (N) of the same oocytes contain differently condensed chromatin regions. (C) Merged image combining (A) and (B). The arrows show the chromatin spots in apparent spatial connection to the PCM-1 aggregates. Note: Bar = 10 μm.

Figure 2. Laser scanning confocal microscopy of ovarian section from a 2-day-old mouse. (A) Three types of less discrete PCM-1 structures accompanied by spreading of the reaction over the nuclear surface. The single and the double dispersing aggregates are marked by arrows. The crescent-shaped PCM-1 cap is shown by an arrowhead. (B) Staining of the same region for chromatin reveals homogenous structure of oocyte nuclei (N). (C) Merged image combining (A) and (B). Note: Bar = 10 μm.
bouquet’ structure, observed in early meiotic stages of a wide range of eukaryotes including mammals, is formed of chromosomal telomeres clustering under the nuclear envelope and facilitates synopsis and recombination of homologous chromosomes during meiotic prophase I [19,20]. It is still debated how and why these two structures associate across the nuclear envelope, but such evolutionary conservation of the centrosome—chromatin interaction in early oocytes throughout the vertebrate lineage would be indicative of its functional significance. Elkouby et al. [18] even hypothesize that microtubules nucleated by the still functional centrosome may transmit force across the nuclear envelope to generate the bouquet configuration of chromosomes.

In ovarian sections from 2-day-old mice, the staining pattern for both PCM-1 and chromatin notably differed from that in 1-day-old samples. The PCM-1 label was less discrete. It was spread at some distance along the nuclear surface in a cap-like fashion without encircling the entire nucleus. There was considerable variation between cells. In some oocytes, the initial single or double aggregate was still recognizable, whereas in others, the PCM-1-containing structure was crescent-shaped with uniform appearance (Figure 2A). The oocyte chromatin showed homogeneous appearance. This indicated chromosome decondensation and was interpreted as a sign of the transition to dictyate and first meiotic arrest (Figure 2B). Compared to the day 1 postnatal ovary, these findings showed a substantial change in centrosome and chromatin organization despite the difference of only 24 hours. The more diffuse, cap-like reaction for PCM-1 spread over the nucleus suggested that, at this stage, centrosomes no longer functioned as sites of attraction of PCM-1 granules (centriolar satellites). This dispersal of PCM-1 could be analogous to the diminished PCM-1 activity during meiotic prophase I [19,20]. It is still debated how and why these two structures associate across the nuclear envelope, but such evolutionary conservation of the centrosome—chromatin interaction in early oocytes throughout the vertebrate lineage would be indicative of its functional significance.

In the present study, detected the centriolar satellite protein PCM-1 in prophase I oocytes from developing primordial mouse follicles and described for the first time its association with condensed chromatin. During transition to dictyate in the key period between the 1st and 2nd day of postnatal life, this protein changed its localization from a discrete aggregate to a more diffuse perinuclear cap. This reorganization of PCM-1 was correlated with chromatin decondensation, presumably indicating synchronized regulation or direct communication across the nuclear envelope.

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Disclosure statement

All authors declare no conflict of interest.

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