Transformation of Sperm Nuclei into Metaphase Chromosomes in the Cytoplasm of Maturing Oocytes of the Mouse

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The cytoplasmic mechanisms that initiate and maintain chromosome condensation have been investigated by introducing the nuclei from various cells into the cytoplasm of maturing oocytes. In amphibians, when nuclei taken from somatic cells such as brain cells are transplanted into the cytoplasm of maturing oocytes, the nuclei are rapidly transformed into metaphase chromosomes (1, 2). Further, when sperm nuclei are introduced into maturing oocytes by inseminating them while they are unresponsive to activation stimuli, the sperm chromatin becomes condensed to a metaphase state (3, 4). Similarly, in the mouse, when somatic cells such as follicle cells or thymocytes are fused to maturing oocytes, the oocyte cytoplasm can transform the nuclei into metaphase chromosomes (5, 6). However, there have been no reports of the direct transformation of sperm chromatin into metaphase chromosomes in mammalian oocyte cytoplasm. Therefore, the experiments described below were carried out to elucidate the behavior of sperm nuclei exposed to the cytoplasm of maturing oocytes of the mouse.

In the first experiment, zona-free oocytes of the mouse were inseminated at prometaphase I or metaphase I of meiosis in vitro, and the behavior of the sperm nuclei within the oocytes cytoplasm was examined. If the oocytes were penetrated by up to 3 sperm, maturation continued during subsequent incubation and became arrested at metaphase II. Meanwhile, each sperm nucleus underwent the following changes. First, the chromatin became slightly dispersed. By 6 hr after insemination, this dispersed chromatin had become coalesced a small mass, from which short chromosomal arms later became projected. Between 12 and 18 hr after insemination, each mass of chromatin resolved into 20 discrete metaphase chromosomes.

In contrast, if oocytes were penetrated by 4 to 6 sperm, oocyte meiosis was arrested at metaphase I, and each sperm nucleus was transformed into a small mass of chromatin rather than into metaphase chromosomes. If oocytes were penetrated by more than 6 sperm, the maternal chromosomes became either decondensed or pycnotic, and the sperm nuclei were transformed into larger masses of chromatin. As controls, oocytes containing germinal vesicle (GV) or at metaphase II were inseminated. In the former, no morphological changes in the sperm nucleus were observed if the oocytes were kept in medium containing dibutyryl cyclic AMP. In the latter, when the oocytes were activated, the sperm nucleus was transformed into the male pronucleus. Therefore, it is clear that mouse oocytes develop a cytoplasmic activity during maturation that can transform the highly condensed sperm chromatin into metaphase chromosomes. However, the capacity of an oocyte is limited, such that it can transform a maximum of 3 sperm nuclei into metaphase chromosomes. Furthermore, the presence of more than 6 sperm causes a loss of the ability of the oocyte to maintain the maternal chromosomes in a metaphase state.

In the second experiment, therefore, we investigated the basis of this limited capacity of the oocyte cytoplasm of transforming sperm chromatin into metaphase chromosomes. We found that if the cytoplasmic volume of oocytes was doubled by polyethyleneglycol-mediated cell fusion, up to 5 sperm nuclei, and if it was tripled, up to 8 sperm nuclei could be transformed into metaphase chromosomes. Conversely, if the volume was reduced by bisection of the oocyte following germinal vesicle breakdown (GVBD), no more than 2 sperm nuclei could be
transformed into metaphase chromosomes. Thus, the capacity of the oocyte to transform sperm nuclei into metaphase chromosomes was proportional to its volume.

In the third experiment, the contribution of GV material and non-GV material in the cytoplasm to the chromosome condensation activity was investigated, using anucleate and nucleate fragments of oocytes bisected before GVBD. In the anucleate fragments, sperm chromosome formation never occurred, indicating that the cytoplasmic activity was completely dependent on the presence of GV material. In the nucleate fragments, the capacity to induce sperm chromosome formation was reduced as compared to single oocytes in spite of the fact that the entire GV contents had been retained in these fragments. Based upon these results it is proposed that maturing oocytes of the mouse develop the cytoplasmic activity that can transform sperm nuclei to metaphase chromosomes and that this activity depends both on the material found inside the GV as well as that found outside the GV.

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