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Review article

Coordination of cellular differentiation, polarity, mitosis and meiosis – New findings from early vertebrate oogenesis

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ABSTRACT

A mechanistic dissection of early oocyte differentiation in vertebrates is key to advancing our knowledge of germline development, reproductive biology, the regulation of meiosis, and all of their associated disorders. Recent advances in the field include breakthroughs in the identification of germline stem cells in Medaka, in the cellular architecture of the germline cyst in mice, in a mechanistic dissection of chromosomal pairing and bouquet formation in meiosis in mice, in tracing oocyte symmetry breaking to the chromosomal bouquet of meiosis in zebrafish, and in the biology of the Balbiani body, a universal oocyte granule. Many of the major events in early oogenesis are universally conserved, and some are co-opted for species-specific needs. The chromosomal events of meiosis are of tremendous consequence to gamete formation and have been extensively studied. New light is now being shed on other aspects of early oocyte differentiation, which were traditionally considered outside the scope of meiosis, and their coordination with meiotic events. The emerging theme is of meiosis as a common groundwork for coordinating multifaceted processes of oocyte differentiation. In an accompanying manuscript we describe methods that allowed for investigations in the zebrafish ovary to contribute to these breakthroughs. Here, we review these advances mostly from the zebrafish and mouse. We discuss oogenesis concepts across established model organisms, and construct an inclusive paradigm for early oocyte differentiation in vertebrates.

1. Introduction

Germ cells undergo fascinating processes during their development. Unlike many other tissues where cells perform a collective tissue function, germ cells differentiate in the gonad to perform an individual function. In the case of the oocyte, this single cell contains the building blocks that initiate the early events of embryonic development following fertilization. The oocyte undergoes a dramatic differentiation process (Fig. 1) that begins when germline stem cells divide, giving rise to oogonia, a mitotic precursor cell of the differentiating meiotic oocyte. Oogonia divide incompletely to generate early oocytes that are inter-connected to sister oocytes via cytoplasmic bridges in a germline cyst. Meiosis initiation transforms the oogonia in the cyst into differentiating oocytes that then separate and become surrounded by somatic granulosa cells in the follicle. In the early differentiating oocyte dramatic nuclear events underlie meiosis, while the cytoplasm in most species becomes polarized. Notably, these intracellular processes occur simultaneously with the changes in cellular organization, all while the oocyte significantly grows in volume.

The Drosophila model has provided a major paradigm of early oogenesis in the field. Because of technical challenges in investigating early oogenesis in vertebrates, several fundamental questions have been long standing. However, in recent years many of these challenges have been overcome and an unprecedented view of early vertebrate oogenesis has immerged. We will discuss recent discoveries on the formation and structure of the germline cyst in the mouse and the fish Medaka, the dissection of chromosomal movement mechanisms during chromosome pairing in meiosis from the mouse, and mechanisms of oocyte symmetry breaking from zebrafish that are linked to meiotic chromosome pairing. We also discuss recent breakthroughs in zebrafish, Xenopus, and the mouse in understanding the biology of the Balbiani body, a universal oocyte feature, comparing common themes in its formation and potential species-specific functions. We further discuss the coordination of these multiple aspects of early oocyte differentiation.

We focus on early oogenesis from germline cyst formation through diplotene stages of the early follicle. Emphasis is on zebrafish oogenesis while comparing common themes with other species, and we mostly use the zebrafish oogenesis staging nomenclature. In an accompanying paper, we provide the methods established for investigating the

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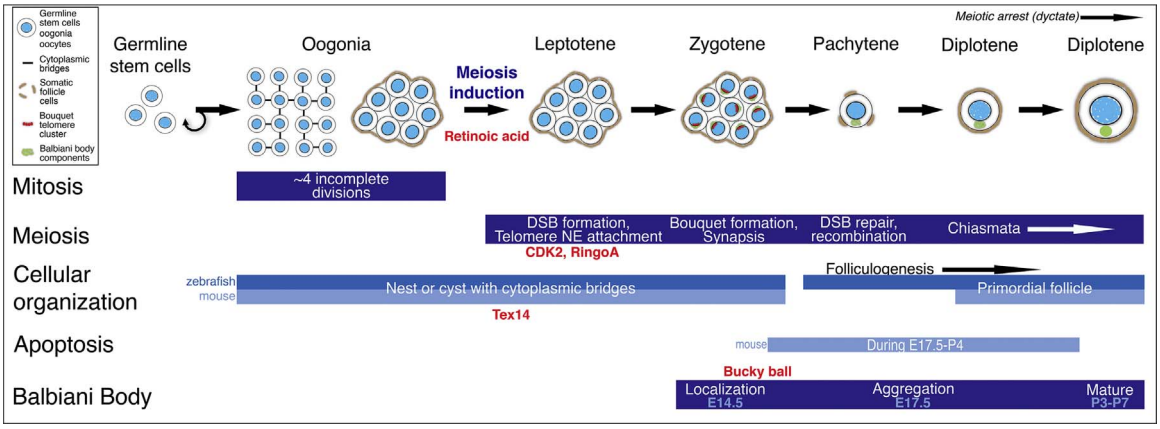

Fig. 1. Early oogenesis in vertebrates. Major events in early oogenesis from germline stem cells through the formation of the primary follicle are depicted. Germline stem cells give rise to the mitotic oogonial cells. Upon the induction of meiosis, oocytes undergo the stages of prophase I: leptotene, zygotene, pachytene and diplotene. Oocytes arrest at diplotene stage (dyctate) until meiosis resumes during oocyte maturation later in oogenesis. The top row shows the cellular organization of oogonial cells, oocytes and somatic follicle cells at each stage. For the oogonia stage, a germline cyst is on the right and a presumptive cell division pattern in the cyst with cytoplasmic bridges depicted is on the left. This division pattern is based on the Drosophila model for germline cyst construction, and remains to be determined in vertebrates. On the left, five major themes in early oogenesis are depicted: mitosis, meiosis, cellular organization, apoptosis, and the Balbiani body mRNP granule. The blue bars to the right of each major process indicate their timing in the oogenesis pipeline. Within the bars, primary events of each process are depicted at each stage. Indigo bars indicate processes that occur similarly in zebrafish and mouse oocytes. Species-specific information is indicated by a dark aqua-blue bar for zebrafish, and a light aqua-blue bar for mouse. Known regulators are indicated in red (CDK2, RingoA and Tex14 were analyzed in mouse oocytes, Bucky ball in zebrafish oocytes. Retinoic acid is a known regulator in mouse and strongly implicated in zebrafish (Rodriguez-Mari et al., 2013)). Telomeres (red) indicate the bouquet configuration at zygotene, and Bb components (green) depict Bb formation in zygotene through diplotene. The proposed scheme provides a conserved unified model for vertebrate oogenesis.

zebrafish ovary that enabled some of the recent zebrafish contributions discussed here.

2. Organization of oogonia and early meiotic oocytes in cysts and nests

2.1. Germline cyst formation and structure

The building blocks of the germline cyst are oogonia, which are mitotically active cells that are born from germline stem cells (GSCs) and give rise to oocytes (Fig. 1). GSCs have recently been identified in the Medaka ovary through lineage tracing studies. It was found that the GSCs localize to what has been called a 'germinal cradle' (Nakamura et al., 2010). These GSCs specifically express the germline marker *nanos2* (Nakamura et al., 2010). Several lines of evidence also indicate that *nanos2* marks the GSCs in the zebrafish. These cells reside in a lateral, anterior-posterior band at the ovary periphery termed the germinal zone (Beer and Draper, 2013). The GSCs in Medaka also reside at the ovary surface but are dispersed along thread-like cords throughout the dorsal surface (Nakamura et al., 2010). Loss of the *nanos2*-expressing cell population in juvenile zebrafish is associated with loss of oocytes and sterility in the adult (Beer and Draper, 2013; Dranow et al., 2013), consistent with *nanos2* marking the GSCs. Future studies are needed to characterize the GSC niche that regulates the production of oogonia from these cells.

Oogonia divide several times in mouse (Lei and Spradling, 2013), frogs (Kloc et al., 2004), Medaka (Nakamura et al., 2010) and zebrafish (Leu and Draper, 2010) (Fig. 1- mitosis), like in Drosophila (Matova and Cooley, 2001; Xie, 2013). During these divisions cytokinesis is incomplete and in the absence of cellular abscission, oogonial cells remain connected via cytoplasmic bridges (CBs) and form a germline cyst (Greenbaum et al., 2007; Kloc et al., 2004; Marlow and Mullins, 2008) (Fig. 1- cellular organization). The germline cyst is engulfed by somatic follicle cells (Elkouby et al., 2016; Leu and Draper, 2010; Nakamura et al., 2010; Pepling, 2012; Selman et al., 1993), and the organization of oogonia and oocytes within germline cysts is highly conserved (Pepling et al., 1999).

The current prevailing model for the oogonial cell division pattern that generates the cyst is the one known from Drosophila. In this model, the cells in the cyst, called cystoblasts, divide 4 times synchronously, giving rise to a 16-cell cyst (Greenbaum et al., 2007; Xie, 2013). In Drosophila, such a pattern is evident by the specialized fusome structure that persists between daughter cells and traces their division planes (Greenbaum et al., 2007; Xie, 2013). A fusome does not form in vertebrates and the pattern and number of divisions of each cell within the cyst has not been addressed. But if the cells develop synchronously, then 2^n cells are expected, where n is the number of cell divisions. Cysts in Xenopus contain up to 16 cells (Kloc et al., 2004), whereas in the Medaka fish and in the mouse, cysts have been identified that are up to 30 or 32 cells, suggesting that an additional round of cell division can occur in the cyst (Lei and Spradling, 2013). Detection of midbodies, a structure of the CB, in clonal meiotic mouse cysts at E14.5 shows that most cells have one or two midbodies and few (~14%) have three or more, but a consistent division pattern or cyst morphology could not be deduced (Lei and Spradling, 2016). Interestingly, the partial breakdown of cysts and their non-clonal aggregation into nests in the mouse demonstrate a cyst-nest aggregation mechanism that is not solely dependent on cell division (Lei and Spradling, 2013), but it is not known if such a mechanism exists in other species.

A prominent feature of the germline cyst is the synchronous development of sister oocytes within it. The intercellular CB connections are thought to facilitate this synchrony through shared cytoplasmic regulators (Pepling et al., 1999). Interestingly, in the re-aggregated nests of clonally unrelated cysts in the mouse, each partial cyst develops synchronously, but not in conjunction with other partial cysts in a nest (Lei and Spradling, 2013). This clonal-specific synchronization supports a CB-mediated synchronization mechanism. CBs assemble on midbodies and require the Tex14 protein (Greenbaum et al., 2009, 2007). Tex14 is required for the construction of the male spermatocyte cyst in the mouse since *tex14*$^{-/-}$ spermatocytes lack CBs, and mutant males are sterile (Greenbaum et al., 2009, 2007, 2006). Tex14 positive midbodies were also detected in cysts at E14.5 through E17.5 in female mice (Greenbaum et al., 2009; Lei and Spradling, 2016). However, *tex14*$^{-/-}$ oocytes lack CBs, but complete oogenesis normally (Greenbaum et al., 2009). While early meiotic stages were not directly addressed, *tex14*$^{-/-}$ females produced developing follicles and were fertile (Greenbaum et al., 2009). This surprisingly demonstrates that CBs, the essence of the cyst structure, are dispensable for oogenesis in the mouse.

A direct examination of the roles of the germline cyst in early