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RNA-binding proteins in human oogenesis: Balancing differentiation and self-renewal in the female fetal germline

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ABSTRACT

Primordial germ cells undergo three significant processes on their path to becoming primary oocytes: the initiation of meiosis, the formation and breakdown of germ cell nests, and the assembly of single oocytes into primordial follicles. However at the onset of meiosis, the germ cell becomes transcriptionally silenced. Consequently translational control of pre-stored mRNAs plays a central role in coordinating gene expression throughout the remainder of oogenesis; RNA binding proteins are key to this regulation. In this review we examine the role of exemplars of such proteins, namely LIN28, DAZL, BOLL and FMRP, and highlight how their roles during germ cell development are critical to oogenesis and the establishment of the primordial follicle pool.

1. Introduction

The finite nature of human female fertility is underpinned by the formation of a non-renewable reserve of primordial follicles that are assembled from mid-gestation onwards in humans (reviewed in (Findlay et al., 2015)). Establishment of the ovarian reserve begins with the migration of primordial germ cells (PGCs) from the proximal epiblast to the genital ridge; a process already underway in the human embryo at four weeks of development (Witschi, 1948; Mollgard et al., 2010), and which is largely complete by the eighth week of gestation (6 weeks post conception) (De Felici, 2013). Upon arrival at the gonad, and following female sex specification, PGCs undergo three significant, overlapping and possibly interconnected processes on their journey to becoming functional oocytes, namely: the initiation of meiosis, the formation and breakdown of germ cell nests, and the assembly of single oocytes into primordial follicles. It is these follicles which constitute the ovarian reserve for the adult life of women, and the developmental events prior to, and during their foundation, that lay the foundations of developmental competence required to form an oocyte that is capable of fertilisation in adult life.

1.1. Forming follicles

The formation of primordial follicles begins around 16 weeks gestation in humans (Motta et al., 1997; Bendsen et al., 2006), as nests of interconnected germ cells break down, releasing individual oocytes to associate with somatic pre-granulosa cells to form primordial follicles. The germ cell nest is an evolutionarily conserved structure, found in males and females from *Drosophila* (de Cuevas et al., 1997) and *Xenopus*, to mice (Pepling et al., 1999) and humans (Motta et al., 1997; Gondos et al., 1971). Nests form as a result of incomplete cytokinesis during germ cell mitosis, leading to the formation of a clonal syncytium of germ cells that divide synchronously and share cytoplasm (Grive and Freiman, 2015). Organelles are exchanged between interconnected germ cells in nests, and their distribution is reorganised just prior to nest breakdown in mice (Pepling and Spradling, 2001), a process linked to the selection of a single oocyte (Lei and Spradling, 2016). Nest breakdown is a coordinated effort involving the loss of germ cells through caspase-dependant apoptosis and physical invasion of the nests by somatic cells (Tingen et al., 2009). It is estimated that up to two-thirds of all germ cells are lost during nest breakdown (Pepling and Spradling, 2001). This culling of germ cells may represent a means of germ cell selection, through which deficient cells are lost and only the highest quality oocytes are assembled into primordial follicles.

In humans, the first primordial follicles to form are located deep within the centre of the fetal ovarian medulla, whilst undifferentiated, mitotic germ cells, with characteristics of PGCs, are found towards the periphery of the ovary (Fig. 1). The human fetal ovary shows distinct

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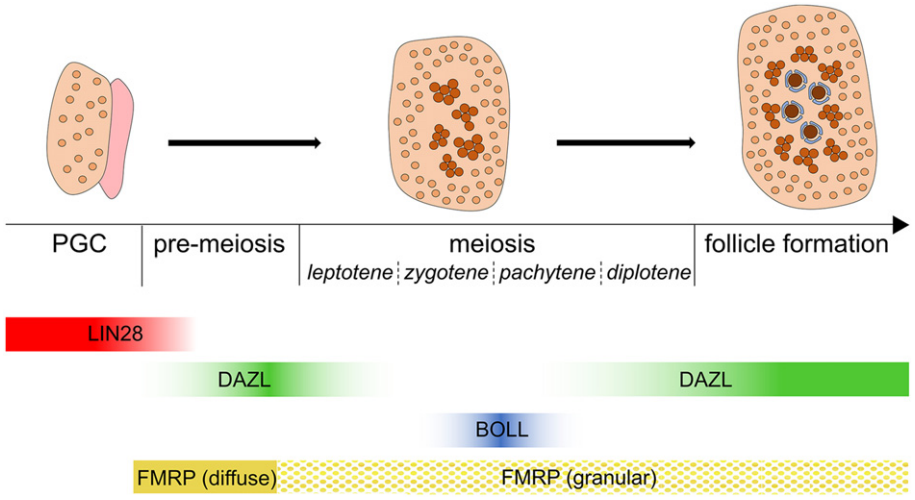

Fig. 1. LIN28, DAZL, BOLL and FMRP expression during germ cell differentiation in females. Cartoon schematic depicts spatial and temporal organisation of germ cells within the human fetal ovary. Germ cells at different stages of maturation are represented by progressively darker shades of orange. LIN28 is present in PGCs. DAZL is expressed before the onset of meiosis but down-regulated afterwards; BOLL is transiently expressed at later stages of meiosis with minimal overlap with DAZL. DAZL is re-expressed in oocytes within primordial follicles. FMRP is present in pre-meiotic germ cells, and yellow dots represent granulation of FMRP staining at the onset of meiosis (Section 2.4).

spatial and temporal organisation, with more differentiated germ cells found progressively deeper into the ovary, establishing a distinct developmental gradient (Anderson et al., 2007; Childs and Anderson, 2012). Thus, the entire developmental spectrum from PGC to primordial follicle can be observed on a single histological section by 18 weeks' gestation, providing an excellent developmental paradigm in which to study the process of cellular differentiation (He et al., 2013a). Similar processes occur in the sheep and cow (Sawyer et al., 2002; Hummitzsch et al., 2013). This cortico-medullary gradient of increasing germ cell differentiation differs from that of the fetal mouse ovary, in which differentiation proceeds in an anterior to posterior (Menke et al., 2003; Bullejos and Koopman, 2004) and possibly dorsal-ventral (Cordeiro et al., 2015) wave along the gonadal axis. Why such differences exist is not clear, but may reflect the need to maintain niches for undifferentiated, proliferating germ cells, which persist alongside more differentiated meiotic germ cells and follicular oocytes in the developing ovaries of larger mammals (Fereydouni et al., 2014; Fulton et al., 2005). In contrast, in the ovaries of feto-neonatal rodents (Kimura et al., 2011), germ cell proliferation is largely complete before the major wave of follicle assembly commences. Despite these differences, however, the assembly of the first follicles occurs at the centre of the developing ovary in both humans and mice, suggesting some aspects of the spatio-temporal regulation of germ cell differentiation may be conserved (Mork et al., 2012; Zheng et al., 2014).

1.2. Meiosis

The initiation of meiosis is one of the defining features of germ cell differentiation, and occurs during fetal life in females, as opposed to from puberty in males. Although comprised of two rounds of cell division, only prophase of meiosis I occurs during fetal oogenesis, with arrest occurring before completion of the first division. The timing of meiotic entry is not intrinsic to germ cells themselves, but rather depends on exposure to retinoic acid produced by the mesonephros in rodents (Bowles et al., 2006; Koubova et al., 2006), but probably by the fetal ovary itself in humans (Childs et al., 2011; Le Bouffant et al., 2010; Bowles et al., 2016; Frydman et al., 2017).

Following pre-meiotic DNA replication, germ cells within nests enter leptotene of prophase I and initiate recombination by generating double strand DNA breaks (Roig et al., 2004; Baudat et al., 2013), leading to the pairing and synapsis of homologous chromosomes during zygotene. The synaptonemal complex, which holds synapsed chromosomes together, is assembled by pachytene, and throughout zygotene and pachytene, meiotic recombination generates crossovers, which not only increase genetic diversity, but also provide physical connections that keep homologous chromosomes together once the synaptonemal complex dissociates in diplotene (Petronczki et al., 2003; MacLennan et al., 2015). Following diplotene, the oocytes enter a period of meiotic (dictyate) and growth arrest, and the nests of interconnected oocytes break down, releasing individual oocytes to form primordial follicles. The oocytes are then maintained in this arrested state until oocyte growth is initiated, a hiatus that can extend to decades in humans. Although oocyte growth occurs throughout follicle development, meiosis only recommences at the time of ovulation. During this prolonged period in stasis, cohesion proteins are important in maintaining the physical linkage between sister chromatids, and deterioration in chromatid cohesion contributes significantly to age-dependent aneuploidy (Jessberger, 2012; Herbert et al., 2015).

Whether germ cell nest breakdown and primordial follicle formation are tied to proper meiotic progression remains unclear. Depletion of synaptonemal complex protein 1 (Sycp1) in fetal rat ovaries (to accelerate the onset of diplotene) resulted in primordial follicles being assembled earlier and in greater numbers than in control ovaries, suggesting an intricate relationship between diplotene arrest and primordial follicle formation (Paredes et al., 2005). However, the ovaries of *Stra8*$^{-/-}$ mice (in which germ cells fail to initiate meiosis) contain 'oocyte-like' cells and follicular structures, suggesting that meiosis and oogenesis/follicle formation may be uncoupled, although the failure of such oocyte-like cells to support development confirms that meiosis is essential to confer reproductive potential (Dokshin et al., 2013; Baltus et al., 2006).

2. RNA-binding proteins in fetal oogenesis

Mammalian gametogenesis, and particularly oogenesis, is punctuated by periods of transcriptional silencing, during which homeostasis and development are dependent on the translation of pre-transcribed mRNAs, under the regulation of RNA-binding proteins (RBPs) (Clarke, 2012; Seydoux and Braun, 2006; Radford et al., 2008). RBPs are an extensive class of proteins, defined by their ability to recognise particular motifs and bind RNA *via* specific recognition sites usually found in 3′ untranslated regions (3′UTRs). RBPs found in the cell nucleus primarily govern nascent mRNA (pre-mRNA) processing events (capping, polyadenylation and splicing), whilst those located in the cytoplasm are known to regulate translation by directing mRNA transport and regulating mRNA stability (Brook et al., 2009). Importantly, RBPs are highly expressed during oogenesis and have been well documented as being