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Review

Control of mammalian germ cell entry into meiosis

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ABSTRACT

Germ cells are unique in undergoing meiosis to generate oocytes and sperm. In mammals, meiosis onset is before birth in females, or at puberty in males, and recent studies have uncovered several regulatory steps involved in initiating meiosis in each sex. Evidence suggests that retinoic acid (RA) induces expression of the critical pre-meiosis gene *Stra8* in germ cells of the fetal ovary, pubertal testis and adult testis. In the fetal testis, CYP26B1 degrades RA, while FGF9 further antagonises RA signalling to suppress meiosis. Failsafe mechanisms involving *Nanos2* may further suppress meiosis in the fetal testis. Here, we draw together the growing knowledge relating to these meiotic control mechanisms, and present evidence that they are co-ordinately regulated and that additional factors remain to be identified. Understanding this regulatory network will illuminate not only how the foundations of mammalian reproduction are laid, but also how mis-regulation of these steps can result in infertility or germline tumours.

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1. Introduction

Successful fertilisation of an oocyte by a sperm brings together two distinct parental genomes and marks the beginning of the next generation. Critical preludes to fertilisation are the formation of a

Abbreviations: dpc, days *post coitum*; DSB, double stranded break; LBD, ligand binding domain; PGC, primordial germ cell; RA, retinoic acid; RARE, retinoic acid response element; TGCT, testicular germ cell tumour(s); TSS, transcription start site; VAD, Vitamin A deficient.

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lineage of specialised cells called germ cells, the precursors of sperm in males and oocytes in females, and the successful progression of meiosis, the special class of germ cell division that yields haploid gametes. While segregation of the germ cell lineage has been studied extensively from a molecular standpoint, and the progression of meiosis is now reasonably well understood, it is only recently that studies have begun to uncover the complex mechanisms by which germ cells choose to embark on meiotic cell division.

Meiosis in mammals begins during fetal life in females, and during puberty in males. It has long been established that the timing of entry into meiosis is not a function intrinsic to the germ cells themselves, but a result of exposure to signals from ovarian or testicular somatic cells. Understanding how entry into meiosis is regulated boils down to discovering the factor(s) present in the fetal ovary, but absent from fetal testes, that are responsible for stimulating germ cells to enter meiosis, and the factor(s) that eventually emerge in the postnatal testis to perform a similar function. Much of our current understanding of these factors has come from studies involving mice, due to the experimental accessibility of this species. In this review we examine the molecular mechanisms revealed to date by studies in mice and confirmed, in many cases, by comparative studies in other mammalian species including humans. We also highlight the current gaps that remain to be investigated.

2. Cellular mechanisms of meiosis

One of the major hallmarks of germ cells is their unique ability to halve their genome through meiosis, thus generating haploid gametes. This process follows a similar chain of events in males and females, despite the dramatically different cellular products in each sex (i.e. sperm or oocytes, respectively). Indeed, general mechanisms of meiosis are remarkably well conserved throughout eukaryotic organisms, from humans to yeast.

Following a round of pre-meiotic DNA replication, meiosis begins and occurs in two parts, meiosis I and II, with each part consisting of four major phases. The first, prophase I, involves the condensation and pairing of homologous chromosomes followed by recombination, allowing for some paternal and some maternal genetic material to be incorporated onto the same chromosome. During subsequent meiotic phases, metaphase I and anaphase I, chromosome pairs are separated by a mechanism involving microtubules attached to centrioles and kinetochores. Finally, during telophase I, the chromosomes reach their respective poles of the cell, and the cell membrane then pinches together to generate two separated cells. The major phases in meiosis II are similar to their meiosis I counterparts, but meiosis II is not preceded by a round of DNA replication, and homologous recombination does not occur during prophase II. As a result meiotic cell division yields four cells from each parental germ cell, each with half the genetic content of the starting cell.

In mice, the first detectable sign of a germ cell's decision to enter meiosis is expression of the gene *stimulated by retinoic acid gene 8* (*Stra8*) which encodes a protein required for pre-meiotic DNA replication and subsequent entry into prophase I (Baltus et al., 2006). Shortly thereafter, expression of genes such as *synaptonemal complex protein 3* (*Sycp3*) and *yeast meiotic recombination protein REC8 homolog* (*Rec8*), encoding proteins involved in the formation of meiotic synaptonemal and cohesion complexes respectively, is observed. The loading of SYCP3 and REC8 proteins to the chromosomes marks the beginning of prophase I as does expression of genes such as *sporulation protein, meiosis-specific, SPO11 homolog* (*Spo11*) and *dosage suppressor of mck1 homolog, meiosis-specific homologous recombination* (*Dmc1*), involved in formation of double stranded breaks (DSB) and homologous recombination. With the formation of DSBs comes the modification of H2A histone family member X (H2AX) to the phosphorylated form, γH2AX, at the site of DNA breaks. This suite of genes and proteins not only provides mechanistic insights into how meiosis works, but also provides useful markers to assess the initiation of meiotic entry during germ cell development, as illustrated in the following sections.

3. Early development of the germ cell lineage

Remarkably, for a population of cells on which reproduction and the propagation of species so critically depends, germ cells do not enjoy a sheltered existence during their life cycle (Fig. 1). At about 6.25 days *post coitum* (dpc) in mice, bone morphogenetic protein (BMP) signalling designates a region of the epiblast that nurtures the formation of primordial germ cell (PGC) precursors (Lawson et al., 1999; Ying et al., 2000; Ying and Zhao, 2001). This involves suppressing somatic cell gene expression and promoting expression of pluripotent genes such as *POU domain, class 5, transcription factor 1* (*Pou5f1* also known as *Oct3/4), Nanog homeobox* (*Nanog*) and *SRY-box containing gene 2* (*Sox2*) (Avilion et al., 2003; Chambers et al., 2007; Scholer et al., 1990). After extensive reprogramming, a mere 40 or so PGCs, marked by the re-expression of *developmental pluripotency-associated 3* (*Dppa3*; also known as *stella* or *Pgc7*), emerge to occupy the proximal epiblast by 7.5 dpc (Saitou et al., 2002; Sato et al., 2002).

The small cohort of PGCs rapidly proliferates and embarks on a two-day migration through the posterior primitive streak, along the elongating hindgut endoderm, to eventually colonise the genital ridges, the primordial gonads (Ginsburg et al., 1990; Molyneaux et al., 2001). Upon entry into the genital ridges, the PGCs adopt a large, rounded morphology and, after epigenomic reprogramming, they become susceptible to differentiation cues from surrounding somatic tissue, which is also developing rapidly at this time (for a review see, Ewen and Koopman, 2010).

The genital ridges are sexually ambiguous, but in XY embryos a subset of somatic genital ridge cells start expressing the male fate determination gene, *sex determining region of Chr Y* (*Sry*), at 10.5 dpc (Koopman et al., 1990; Sekido et al., 2004), priming the self-maintaining expression of *SRY-box containing gene 9* (*Sox9*) to drive the gonad towards testis development (Kim et al., 2006; Koopman et al., 1991; Sekido et al., 2004). Following the differentiation of Sertoli cells, which surround and provide support to the germ cells, other cell types form, including steroidogenic Leydig cells. In the absence of *Sry*, wingless-related MMTV integration site (WNT) signalling through β-catenin, together with *forkhead box L2* (*Foxl2*) expression in the XX somatic cells, steer the gonads to develop into ovaries (Kashimada et al., 2011a; Kim et al., 2006). Pre-granulosa cells, the female counterpart of Sertoli cells, provide support to XX germ cells, and steroidogenic theca cells form. Throughout this early period germ cells remain bipotential: XX germ cells between 11.5 and 12.5 dpc are capable of developing into prospermatogonia when cultured together with 12.5 dpc XY genital ridge cells (Adams and McLaren, 2002), while 11.5 dpc XY germ cells are triggered to enter meiosis and exhibit condensed chromatin staining when co-cultured in the presence of XX somatic tissue (Adams and McLaren, 2002; Byskov and Saxen, 1976). These disaggregation/reaggregation experiments suggest that, irrespective of their genetic makeup, the environmental context in which the germ cells reside determines which sexual path they follow.

4. The beginnings of meiosis

Table 1 lists some of the genes associated with meiotic entry in mice. In the developing ovary, between 12.5 and 16.5 dpc, germ