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Meiosis and retrotransposon silencing during germ cell development in mice

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Abstract

In mammals, germ cells derive from the pluripotent cells that are present early in embryogenesis, and then differentiate into male sperm or female eggs as development proceeds. Fusion between an egg and a sperm at fertilisation allows genetic information from both parents to be transmitted to the next generation, and produces a pluripotent zygote to initiate the next round of embryogenesis. Meiosis is a central event in this self-perpetuating cycle that creates genetic diversity by generating new combinations of existing genetic alleles, and halves the number of chromosomes in the developing male and female germ cells to allow chromosome number to be maintained through successive generations. The developing germ cells also help to maintain genetic and chromosomal stability through the generations by protecting the genome from excessive de novo mutation. Several mouse mutants have recently been characterised whose germ cells exhibit defects in silencing the potentially mutagenic endogenous retroviruses and other retrotransposons that are prevalent in mammalian genomes, and these germ cells also exhibit defects in progression through meiosis. Here we review how mouse germ cells develop and proceed through meiosis, how mouse germ cells silence endogenous retroviruses and other retrotransposons, and discuss why silencing of endogenous retroviruses and other retrotransposons may be required for meiotic progression in mice.

Germ Cell Development in Mice

As genetic information in mammals is transmitted through successive generations it is passed in a self-perpetuating cycle from pluripotent cells in the early embryo to germ cells and back again (Figure 1). This germline cycle is at the core of the mammalian life cycle, and any changes or mutations that are introduced into the genetic material during this cycle have the potential to be passed on to subsequent generations. Germ cell development is initiated around a third of the way through embryogenesis in mice and continues through to adulthood (Figure 2) [1,2,3]. There are no germ cells present in mouse embryos during the early stages of development, but the pluripotent cells that are present at these stages have the capacity to differentiate all the cell types present in the developing embryo including the germ cells. Germ cell specification occurs at around 6.0-6.5 days post coitum (dpc) when a small number of pluripotent cells in the post-implantation epiblast differentiate into primordial germ cells [4]. The primordial germ cells then proliferate and migrate through the developing embryo, arriving at the emerging gonad around 10.5 dpc. Once the germ cells colonise the gonad, their development starts to be directed and regulated by signals originating from gonadal somatic cells, particularly the supporting cell lineage that will differentiate into male Sertoli cells or female granulosa cells [5]. At around 11.5 dpc the proliferating germ cells undergo an extensive reprogramming event that alters the DNA methylation and histone modification status of their chromatin [6,7]. These changes erase some of the epigenetic marks associated with imprinted genes and presumably help prevent the germ cells from transmitting inappropriate epigenetic information between generations. At 12.5-13.5 dpc primordial germ cells stop proliferating and enter a transient post-mitotic/pre-meiotic state, probably in response to an intrinsic timing mechanism. The primordial germ cells are then directed to differentiate down either a male or a female developmental pathway by signals emanating from the somatic cells in the foetal gonad environment [8,9]. If the germ cells decide to embark down a female oogenic pathway, the primordial germ cells initiate meiosis en masse in the foetal ovary, progress through early meiotic prophase, and arrest at the diplotene stage of meiosis as dictyate oocytes a few days after birth. The

ovarian somatic environment appears to be involved in regulating oocyte growth and maturation to generate a controlled release of oocytes throughout the reproductive life-span of the adult female. Cohorts of oocytes are selected for growth and maturation in response to hormonal stimulation, and maternal imprints and de novo DNA methylation marks are established on the chromatin in growing oocytes [10]. Meiosis resumes in mature oocytes just prior to ovulation, and results in the oocyte dividing asymmetrically to generate a large secondary oocyte, or egg, and a small polar body. Meiosis is arrested at the second meiotic metaphase in mature eggs, and completion of meiosis and extrusion of the second polar body does not occur until the egg is fertilised.

In contrast if the primordial germ cells in the foetal gonad decide to embark on a male spermatogenic pathway, the germ cells become enclosed in testis cords along with the Sertoli cells. Depending on the mouse strain [11], the male prospermatogonia may undergo a small number of mitotic divisions before entering a period of quiescence that lasts until a few days after birth. During this period of quiescence, paternal imprints and de novo DNA methylation marks are established on the germ cell chromatin [12,13]. Mitosis resumes a few days after birth, and some of the prospermatogonia differentiate into spermatogonial stem cells, while others directly give rise to differentiated A-type spermatogonia that continue to develop down the spermatogenic pathway. As a result, the first wave of spermatogenesis in prepubertal mice is unique in that the differentiating germ cells have not passed through the spermatogonial stem cell compartment [14]. The spermatogonial stem cells provide a constant supply of differentiated A-type spermatogonia throughout the reproductive life-span of the adult male, with signals from the Sertoli cells and other somatic cells in the testis regulating the production of differentiated A-type spermatogonia. The differentiated A-type spermatogonia undergo a set number of mitotic divisions and further morphological differentiation until they become preleptotene spermatocytes and initiate meiosis. Meiosis proceeds without interruption in the male spermatocytes to produce haploid round spermatids. The round spermatids then undergo substantial morphological changes to become

elongated spermatids and finally mature spermatozoa. The germ cell chromatin undergoes major changes in the round and elongated spermatids as histones are replaced first by transition proteins then by protamines to achieve the high levels of DNA compaction evident in mature sperm. After fertilisation, protamines associated with the paternal DNA are replaced by histones, and the paternal DNA is extensively demethylated before the maternal and paternal genomes fuse together in the zygote [15].

Chromosome behaviour during meiosis

As can be seen from the previous section, meiosis is a central event in the differentiation of both male and female germ cells. Meiosis reduces the chromosome content of diploid cells, which contain two homologous copies of each chromosome, to allow the generation of haploid gametes containing one copy of each chromosome. During meiosis, haploid cells are generated by coupling one round of DNA replication to two rounds of chromosome segregation and cell division (Figure 3) [16]. The first round of chromosome segregation, meiosis I, is a reductional division where only one of the two homologs of each duplicated chromosome is partitioned into each of the two daughter cells. The second round of chromosome segregation, meiosis II, is an equational division where the duplicated chromosomes separate into their constituent sister chromatids that partition equally between two daughter cells. The elaborate behaviour of the chromosomes during meiosis, particularly during meiosis I, is remarkably conserved throughout eukaryotic species, and reflects the meiotic chromosomes condensing, pairing, recombining and synapsing. Many of these interdependent chromosomal events are readily detectable by light microscopy and are often used to subdivide meiosis I into discrete stages (Figure 3). The chromosome condensation, pairing, recombination and synapsis that occur during meiosis are important for correct chromosome segregation during meiosis and ensure that a unique haploid set of chromosomes is present in each mature gamete.

Premeiotic DNA replication

The first step in progression through meiosis is premeiotic DNA replication. Premeiotic DNA replication occurs in the preleptotene stage of meiosis, but the intracellular and extracellular signals involved in regulating the initiation of premeiotic DNA replication in developing germ cells are poorly understood [5]. Premeiotic DNA replication itself is likely to be mostly carried out by many of the same molecules and enzymes involved in replicating DNA in mitotic cells. However in many species premeiotic DNA replication takes considerably longer than mitotic DNA replication suggesting that there are some differences between these processes [17]. The assembly of meiosis-

specific cohesins that help to generate the correct chromosomal architecture for meiosis is initiated during premeiotic DNA replication [18]. Thus processes such as assembly of meiosis-specific cohesins that are initiated during premeiotic DNA replication are probably important for subsequent meiotic chromosome behaviour and, at least in yeast, cells must undergo premeiotic rather than mitotic DNA replication for subsequent meiotic chromosome segregation to occur correctly [19].

Meiosis-specific cohesins

The meiotic recombination, chromosome pairing and chromosome synapsis that follow premeiotic DNA replication demand a specific chromosomal architecture that includes compacting the chromosomes and arranging the chromatin into loops that extend from a central axis. There is some specificity in the sequences that associate with the loops and axes [20], which may have some influence on which chromosomal sequences can participate in some of the later meiotic events. The organization of meiotic chromosomes into these structures depends at least in part on meiosis-specific cohesin proteins [21]. Cohesins play an important role in chromosome structure and in maintaining connections between sister chromatids in meiosis and mitosis. In mitosis the cohesin complex consists of four subunits, Smc1, Smc3, Scc1 and Scc3, and maintains connections between the two newly replicated sister chromatids. At the mitotic metaphase-anaphase transition, cleavage of Scc3 throughout the entire chromosome releases the sister chromatids for segregation. In contrast, the incorporation of meiosis-specific cohesin subunits Smc1β, Rec8, and Stag3 into meiotic chromosomes is at least partly responsible for the way that chromosomes segregate during the two meiotic divisions. During meiosis I when homologous chromosomes rather than sister chromatids are being segregated, cohesin complexes are cleaved along the chromosome arms but not at the centromeres. Cohesin cleavage along the chromosome arms is thought to allow the chiasmata that link the homologous chromosomes to resolve and the homologous chromosomes to separate during the metaphase-anaphase transition of meiosis I. The persistence of cohesin complexes at the centromeres during meiosis I ensures that the physical connection between sister chromatids is maintained at this stage. During the metaphase-anaphase transition of meiosis II, the

centromeric cohesin complexes are cleaved to allow the separation and segregation of sister chromatids. Thus the incorporation of meiosis-specific cohesins into chromosomes is important for establishing the structure and segregation pattern of the chromosomes during meiosis.

Knock-out mice that lack meiosis-specific cohesin subunits have specific phenotypes that reflect the importance of establishing the correct chromosomal architecture during meiosis. For example in *Rec8*$^{-/-}$ and *Smc1β*$^{-/-}$ knockout mice, the meiotic chromosomes are highly reduced in length as a result of an increase in the size of the chromatin loops that extend from the chromosome axis [22,23]. Additionally, in *Smc1β*$^{-/-}$ mice meiotic recombination and homologous chromosome synapsis are impaired, presumably as a consequence of the disruption to meiotic chromosome structure, and sister chromatid cohesion is lost prematurely [22]. Meiosis arrests during meiosis I in *Smc1β*$^{-/-}$ male germ cells, and during meiosis II in *Smc1β*$^{-/-}$ female germ cells leading to sterility in both sexes [22]. Similar problems arise in *Rec8*$^{-/-}$ knockout mice where synapsis and recombination between homologous chromosomes are impaired leading to sterility in both sexes [23,24]. Interestingly, synapsis and recombination occur between sister chromatids rather than between homologous chromosomes in *Rec8*$^{-/-}$ mice [24]. Abnormalities in meiotic chromosome structure presumably underlie the aberrant recombination and synapsis in *Rec8*$^{-/-}$ and *Smc1β*$^{-/-}$ germ cells.

Meiotic recombination

Meiotic recombination is important for generating genetic diversity in the gametes, and for generating the correct pattern of chromosome segregation during meiosis. Recombination between homologous chromosomes is initiated during the leptotene stage of meiosis when the meiosis-specific Spo11 endonuclease introduces several hundred DNA double strand breaks into the genome [25,26]. The appearance of these DNA double-strand breaks can be monitored by immunostaining for the modified histone γH2AX, and by the assembly of early recombination foci containing the Rad51 recombinase and its meiosis-specific paralog Dmc1 on the meiotic chromosome axes. Processing of the DNA double-strand breaks at these early recombination foci is thought to generate

single stranded DNA ends that invade adjacent DNA molecules to search for homologous DNA sequences. Interaction between these invasion intermediates and the second DNA double strand break end results in the formation of a meiotic recombination intermediate known as a double Holliday junction that possesses regions of heteroduplex DNA containing complementary DNA strands from the two homologous chromosomes. These double Holliday junctions are resolved by cutting and religation of two of the four DNA strands. Depending on which strands are cut and re-ligated, the double Holliday junction will be resolved into either a non-crossover event where only small regions of single DNA strands have been exchanged between chromatids from homologous chromosomes, or a crossover event where both DNA strands of the recombining chromatids are exchanged between homologous chromosomes at the recombination site. These crossover events appear as chiasmata that maintain the physical connection between homologous chromosomes after the synaptonemal complex disassembles later in meiosis I. In both crossover and non-crossover events, early recombination markers such as Dmc1 and Rad51 are lost from meiotic chromosomes as the recombination sites mature and incorporate the single-stranded DNA binding protein RPA and the recombination-associated protein Msh4. Incorporation of the recombination-associated protein Mlh1 into late recombination sites marks the formation of crossover events. The number of recombination sites in the nucleus decreases from a few hundred early recombination sites to around twenty-five Mlh1-containing crossovers as meiosis proceeds [27]. Chiasmata are involved in maintaining the physical connection between homologous chromosomes during meiosis I, therefore each of the twenty mouse autosomal chromosomes has to develop at least one chiasma to ensure accurate meiotic chromosome segregation. The mechanisms that regulate the number and distribution of chiasmata in the meiotic genome are still currently poorly understood.

Mice that lack genes involved in the early steps of meiotic recombination, such as *Spo11* or *Dmc1*, are infertile with the meiotic germ cells failing to pair and synapse homologous chromosomes [28,29,30]. Interestingly, although *Spo11*$^{-/-}$ knockout mice do not generate Rad51/Dmc1-containing early recombination sites, treating these mice with the cisplatin, a DNA damaging agent that

introduces DNA double-strand breaks, results in the appearance of Rad51/Dmc1-containing early recombination sites and partially rescues the *Spo11*$^{-/-}$ phenotype [29]. The failure in homologous chromosome pairing and synapsis in *Spo11*$^{-/-}$ and *Dmc1*$^{-/-}$ knockout mice presumably reflects the involvement of early meiotic recombination in the homology search. Conversely, homologous chromosome pairing and synapsis appear to be required for early recombination sites to mature and for meiotic recombination to proceed [31] suggesting that the progression of meiotic recombination is closely interdependent with other chromosomal events in meiosis. Furthermore mutations in *Mlh1*, which is involved in the late stages of recombination do not disrupt homologous chromosome pairing and synapsis but result in the formation of achiasmate meiotic chromosomes that do not segregate correctly during meiosis I [32,33]. Thus defects in meiotic recombination can influence many of the other chromosomal events that occur during meiosis.

Homologous chromosome pairing and synapsis

The process of aligning homologous chromosomes during the zygotene-pachytene stages of meiosis I typically occurs in two phases. First during homologous chromosome pairing, chromosomes find their homologues and roughly align their axes in the absence of any obvious electron-dense physical connection. Then during homologous chromosome synapsis, the proteinaceous synaptonemal complex structure assembles between homologous chromosomes bringing the chromosomal axes closer together [26]. Although homologous chromosome pairing and synapsis can be readily distinguished in many species, homologous chromosome alignment in mice appears to occur segmentally with both pairing and synapsis taking place at the same time in the nucleus. It is not clear at present how each chromosome searches for and identifies its homolog, or which sequences on each chromosome are involved in the homology search. Indeed, the mechanism of homologous chromosome pairing appears to differ between species. In *C.elegans* and *Drosophila*, homologous chromosome pairing occurs independently from the DNA double strand breaks generated during early meiotic recombination and is attributed to recognition of centromeres and specific chromosomal regions. In contrast, homologous chromosome pairing in mice depends on the DNA

double strand breaks and early meiotic recombination sites that are normally generated by Spo11 [28,29]. In *Spo11*$^{-/-}$ mice, and in other mouse mutants with defects in homolog pairing, some synaptonemal complex assembly does eventually occur between non-homologous chromosomes suggesting that paired homologous chromosomes might be preferred but not required for synaptonemal complex assembly.

Assembly of the synaptonemal complex is initiated during the leptotene stage of meiosis, prior to homologous chromosome pairing, when the axial elements of the synaptonemal complex assemble on the meiotic chromosomal axes [34]. The assembly of the Sycp1 transverse filament protein into the synaptonemal complex mediates chromosome synapsis by providing a zipper-like connection between axial elements from homologous chromosomes. As synapsis proceeds during zygotene the axial elements become the lateral elements of the synaptonemal complex, connected to a central element structure by Sycp1-containing transverse filaments. Synapsis is complete by the pachytene stage of meiosis and results in the synaptonemal complex spanning a ~100 nm gap between the axes of two homologous chromosomes. The size of the gap between the two chromosomal axes is primarily determined by the length of the central coiled-coil domain in Sycp1 [35]. The Sycp1 protein molecules are arranged with their C-termini anchored in the lateral elements and their N-termini located in a central element where they interact with the central element components Syce1, Syce2 and Tex12 [36,37]. Thus assembly of Sycp1 or central element proteins into the synaptonemal complex can be used to monitor chromosome synapsis during meiosis.

Interestingly, mice that lack genes encoding either of the lateral element components *Sycp2* or *Sycp3* exhibit sexually dimorphic phenotypes [38,39,40]. Male *Sycp2*$^{-/-}$ or *Sycp3*$^{-/-}$ knockout mice are unable to form lateral elements, have impaired meiotic chromosome synapsis, and are infertile due to germ cell undergoing apoptosis at the pachytene stage of meiosis [38,40]. Some assembly of Sycp1 filaments does occur in the absence of axial elements in *Sycp2*$^{-/-}$ and *Sycp3*$^{-/-}$ mice, indicating that Sycp1 may be able to interact with other proteins or DNA present in the meiotic chromosomal

axes. However the Sycp1 filaments are somewhat disordered in *Sycp2*$^{-/-}$ and *Sycp3*$^{-/-}$ mice and do not appear to be completely functional. Assembly of the axial element of the synaptonemal complex may also influence chromosome structure or condensation as *Sycp3*$^{-/-}$ knockout spermatocytes have meiotic chromosomes with longer chromosome axes than normal [41]. However, early meiotic recombination and pairing between homologous chromosomes are not impaired in *Sycp3*$^{-/-}$ spermatocytes [40,42]. Although these problems lead to infertility in male mice, *Sycp2*$^{-/-}$ and *Sycp3*$^{-/-}$ female mice are fertile, albeit at a reduced level [38,39]. In *Sycp3*$^{-/-}$ mice the reduced level of fertility observed in females is, at least in part, due to a failure in chiasmata formation and the resulting high rates of aneuploidy in the mature female gametes [39].

In contrast, in the absence of *Sycp1* homologous chromosomes pair along their entire axes, but chromosome synapsis does not occur [31]. The lack of synapsis in *Sycp1*$^{-/-}$ mice presumably leads to defects in the appearance of Mlh1-positive late recombination sites and apoptosis at the pachytene stage of meiosis in male mice [31]. The central element proteins are also required for the normal progression of chromosome synapsis and appear to play a role in stabilizing the assembling synaptonemal complex [43,44,45]. In the absence of central element proteins, short regions of synaptonemal complex assemble but are unable to extend along the entire chromosome axis. The association between these short stretches of synaptonemal complex and meiotic recombination sites may reflect synaptonemal complex assembly preferentially initiating at sites of meiotic recombination [45]. This further illustrates the interdependence between meiotic recombination and homologous chromosome pairing and synapsis during meiosis: both homolog pairing and chromosome synapsis appear to be initiated by meiotic recombination, while successful homolog pairing and synapsis allows meiotic recombination to proceed. Once the synaptonemal complex disassembles at the diplotene stage of meiosis, the physical connection between homologous chromosomes is maintained by the chiasmata and by cohesin complexes linking the sister chromatid arms. These homologous chromosome pairs can then align on the meiotic spindle for reductional segregation in meiosis I.

In male germ cells the X and Y sex chromosomes behave somewhat differently to the autosomal chromosomes during meiosis [46]. The limited sequence homology between the X and Y chromosomes means that synapsis between the sex chromosomes is restricted to the relatively small pseudoautosomal region, and markers of double-strand DNA breaks and early recombination proteins persist on the non-recombining regions of the sex chromosomes that lie outside this pseudoautosomal region. The X and Y chromosomes also acquire repressive histone modifications, become transcriptionally silent, and form a distinct structure known as the XY-body during pachytene. This process of meiotic sex chromosome inactivation may be a cellular response to the presence of unsynapsed DNA in the pachytene stage of meiosis, and may help prevent the unsynapsed sex chromosome DNA from activating a pachytene checkpoint monitoring synapsis.

Detecting aberrant meiotic chromosome behaviour

Both male and female germ cells possess meiotic checkpoint mechanisms that detect aberrant chromosome behaviour during meiosis and help prevent the formation of aneuploid gametes. In male mice, defects in meiotic chromosome behaviour tend to result in germ cell death during spermatogenesis, often during the mid-pachytene stage of meiosis. Mutations in a range of different genes encoding meiotic cohesion subunits, early recombination proteins or components of the synaptonemal complex all appear to trigger spermatocyte apoptosis during pachytene [47], and a recurring feature in these knockout mice is that the mutant pachytene spermatocytes exhibit some degree of chromosome asynapsis and contain DNA double strand breaks derived from unresolved early recombination sites. Therefore it is possible that a pachytene checkpoint in male mice is monitoring synapsis by triggering apoptosis in response to the presence of DNA double strand breaks or stalled recombination intermediates, in a manner analogous to the DNA damage checkpoints operating during the mitotic cell cycle [48]. Alternatively, the asynaptic chromosomes may sequester components of the meiotic sex chromosome inactivation machinery and the resulting

ectopic silencing of autosomal gene expression, or the failure to adequately silence gene expression from the X and Y sex chromosomes, may cause inappropriate gene expression during pachytene and apoptosis [49]. There is evidence from *Spo11*$^{-/-}$ knockout mice that asynaptic chromosomes can trigger pachytene spermatocyte apoptosis in the absence of meiotic DNA double-strand breaks [28,29], and there is also evidence that defects in meiotic recombination can trigger pachytene spermatocyte apoptosis in the absence of chromosome asynapsis or defective meiotic sex chromosome inactivation [50]. Thus the pachytene spermatocyte's strong response to asynaptic chromosomes may reflect the existence of multiple mechanisms that can respond to defects in meiotic progression.

Pachytene stage female meiotic germ cells also appear to monitor chromosome synapsis through recognizing unresolved DNA double strand breaks or stalled recombination intermediates [50,51]. Female oocytes probably also possess a DNA double strand break independent mechanism for monitoring asynapsis, such as transcriptional silencing of unsynapsed chromosomes, as asynaptic female oocytes undergo apoptosis in *Spo11*$^{-/-}$ knockout mice that lack meiotic DNA double strand breaks [28,29,51]. However, in contrast to male XY spermatocytes, female XX oocytes do not undergo meiotic sex chromosome inactivation, and therefore failure of meiotic sex chromosome inactivation does not contribute to the apoptosis of asynaptic pachytene oocytes. Indeed disruption of the male-specific meiotic sex chromosome inactivation process may be responsible for the greater sensitivity of male germ cells to asynaptic pachytene chromosomes and presumably contributes to the sexual dimorphism that has been reported in some mouse meiotic mutant phenotypes [49,52]. For example, mice lacking the synaptonemal complex protein Sycp3 exhibit defective recombination and synapsis in both male and female meiotic germ cells, but although *Sycp3*$^{-/-}$ knockout pachytene spermatocytes undergo apoptosis resulting in male infertility, at least some *Sycp3*$^{-/-}$ knockout oocytes can proceed through meiosis resulting in an increased frequency of aneuploidy and female subfertility [39,40]. Additional sex-specific differences in post-pachytene

checkpoint mechanisms monitoring the attachment of the homologous chromosome pairs to the meiotic spindle probably also contribute to the sexually dimorphic phenotypes of some mouse meiotic mutants [49,52].

Retrotransposon silencing in the mouse germline

In recent years a number of mouse mutants have been generated that have defects in silencing retrotransposons in the developing germline. Interestingly many of these mutants also exhibit defects in meiosis suggesting that stable silencing of retrotransposons may be required for progression through meiosis. Retrotransposons are mobile genetic elements that use RNA intermediates to amplify and move themselves to new locations in the genome [53]. Mammalian retrotransposons can be classified into three main categories: long interspersed elements (LINEs; 660,000 copies, ~20% of the mouse genome) are ancient retrotransposons that encode two proteins required to mediate retrotransposition; short interspersed repeats (SINEs; 150,000 copies, ~10% of the mouse genome) are derived from endogenous small cellular RNAs with no protein-coding capacity, and use proteins encoded by LINEs for their retrotransposition; and endogenous retroviruses (ERVs; 630,000 copies, ~10% of the mouse genome) that have a genomic structure related to simple retroviruses with *Gag*, *Pol* and sometimes *Env* protein-coding genes flanked by a long-terminal repeat (LTR). Any successful retrotransposable element is presumably active in germ cells, or early embryonic precursors of germ cells, at some stage of development to allow new retrotransposition events to be propagated through subsequent generations. New retrotransposition events can help to drive genome evolution but are also potentially mutagenic. At least in mice, developing germ cells appear to possess mechanisms to silence expression of retrotransposons and limit their mutagenic activity.

Retrotransposon silencing by DNA methylation

A principle mechanism for silencing expression of retrotransposons in somatic cells is transcriptional gene repression through DNA methylation. DNA methylation can contribute to the silencing of gene expression by preventing access of transcription factors or by recruiting transcriptionally repressive methylated DNA binding proteins and chromatin modifications to genomic loci [54]. In mammalian cells, DNA methylation predominantly occurs on cytosine bases

within CpG dinucleotides and can be catalysed by one of three DNA methyltransferase enzymes: Dnmt1, Dnmt3a or Dnmt3b. Dnmt1 is a maintenance methyltransferase that is primarily responsible for copying methylation patterns from the old DNA strand to the new DNA strand after DNA replication, thereby preserving DNA methylation patterns through successive cell divisions. Dnmt3a and Dnmt3b are de novo methyltransferases that are primarily responsible for establishing new DNA methylation patterns on unmethylated DNA. In addition, *Dnmt3a*$^{-/-}$ *Dnmt3b*$^{-/-}$ double knockout embryonic stem cells exhibit some loss of DNA methylation, suggesting that the de novo methyltransferases help to correct imperfections in Dnmt1-mediated maintenance of DNA methylation [55]. Several lines of evidence suggest that DNA methylation plays a role in silencing retrotransposons. The majority of the DNA methylation in the mammalian genome is thought to be located in retrotransposon sequences and retrotransposons are activated in the event of genome-wide demethylation [56]. Chemical inhibition of DNA methyltransferases with 5-azacytidine results in reduced levels of DNA methylation, an increase in expression of the intracisternal A particle (IAP) endogenous retrovirus mRNA and an increase in production of IAP viral particles in mouse embryonic fibroblasts [57]. Cultured mouse embryonic fibroblasts that carry mutations in *Dnmt1* exhibit genome-wide demethylation and also increase IAP retrotransposon expression around 50-fold [58]. Similarly, *Dnmt1*$^{-/-}$ mutant mouse embryos have significantly reduced levels of DNA methylation and a comparable increase in the level IAP retrotransposon expression in their somatic tissues at 9.5 dpc [59].

DNA methylation appears to play a role in silencing retrotransposons in germ cells as well as somatic cells [60,61]. However, DNA methylation patterns in the germ cells change during development, and differ between the sexes. Germ cells undergo widespread DNA demethylation soon after colonising the gonads resulting in partial demethylation of IAP retrotransposons by 11.5 dpc [6]. In male and female embryos, DNA methylation at IAP elements is further reduced over the next few days, although the kinetics of the loss in DNA methylation differs between the sexes and between different classes of retrotransposons [6,12,59,62]. Subsequent de novo methylation of

retrotransposons occurs at different times in male and female germ cells: IAP elements in the quiescent prospermatogonia in 17.5 dpc male embryos are fully methylated whereas methylation of these elements remains low at this stage in females [12]. De novo methylation of retrotransposons in the female germline occurs in growing oocytes after birth [10].

Dnmt3L is a germ cell specific, catalytically inactive member of the DNA methyltransferase family and acts as a cofactor for the de novo DNA methyltransferases Dnmt3a and Dnmt3b [61,63]. In *Dnmt3L*$^{-/-}$ knockout mice, de novo methylation of IAP and LINE1 retrotransposons does not appear to occur in quiescent prospermatogonia, and IAP and LINE1 elements are expressed in *Dnmt3L*$^{-/-}$ spermatogonia and spermatocytes after birth [61]. *Dnmt3L*$^{-/-}$ knockout spermatocytes initiate meiosis but are not able to progress beyond the pachytene stage. Meiotic recombination appears to be initiated in *Dnmt3L*$^{-/-}$ spermatocytes, and assembly of the axial elements of the synaptonemal complex takes place, but chromosome synapsis is impaired [61,64].

Retransposon silencing by the mouse Piwi-like proteins

In addition to *Dnmt3L*, the mouse *Mili* and *Miwi2* genes are also required for de novo methylation of retrotransposons in quiescent prospermatogonia [62]. *Mili* and *Miwi2* contain a piwi domain that is implicated in binding and cleaving RNA molecules, and are murine orthologs of the Drosophila *piwi* gene involved in silencing retrotransposons in the fly germline [65,66]. Deletion of either *Mili* or *Miwi2* in mice results in male sterility with spermatogenesis failing to progress beyond meiosis [67,68]. Meiotic spermatocytes in *Mili*$^{-/-}$ mutant testes assemble the axial elements of the synaptonemal complex but appear have problems generating fully synapsed pachytene chromosomes and undergo apoptosis during meiotic prophase [67]. Meiotic spermatocytes in *Miwi2*$^{-/-}$ mutant mice are able to initiate meiotic recombination and assemble the axial elements of the synaptonemal complex, but homologous chromosome synapsis is impaired and *Miwi2*$^{-/-}$ meiotic germ cells also undergo apoptosis [68]. Furthermore, like in *Dnmt3L*$^{-/-}$ mutant mice, *Mili*$^{-/-}$ and *Miwi2*$^{-/-}$ neonatal testes accumulate LINE1 and IAP retrotransposon transcripts, although

upregulation of IAP element expression is significantly lower in *Miwi2*$^{-/-}$ mice than *Mili*$^{-/-}$ mice [62,69]. *Miwi2*$^{-/-}$ and *Mili*$^{-/-}$ testes have reduced DNA methylation at LINE1 and IAP elements, and the DNA methylation defect seen in *Mili*$^{-/-}$ mice appears to result from impaired de novo methylation of LINE1 and IAP elements in quiescent prospermatogonia [62,69]. This suggests that the mouse Piwi-like proteins are directly or indirectly involved in de novo methylation of retrotransposons in developing male germ cells. Piwi-like proteins are physically associated with small RNA molecules (piRNAs) [70,71], and a large proportion of the piRNAs sequences that are present in the quiescent prospermatogonia at the time of de novo DNA methylation have sequence homology with retrotransposons [62]. It has been suggested that the piRNA/Piwi-like protein complexes serve as sequence-specific guides that direct the de novo DNA methylation machinery to transposable elements in the mouse genome [62,69], but more indirect relationships between piRNA/Piwi-like protein complexes and de novo DNA methylation cannot be excluded at present [72]. It will be clearly be of great interest to determine the mechanistic link between piRNA/Piwi-like protein complexes and de novo DNA methylation of retrotransposon sequences in developing male germ cells.

Loss of the third murine piwi-like protein, Miwi, also results in male infertility and defective spermatogenesis [73], but *Miwi* appears to function later in spermatogenesis than either *Mili* or *Miwi2*. *Miwi* is expressed in meiotic spermatocytes and post-meiotic round spermatids in adult testes but is not expressed in quiescent prospermatogonia in the embryo when de novo methylation of retrotransposons occurs [73,74]. In *Miwi*$^{-/-}$ knockout mice the male germ cells progress through meiosis to become round spermatids, but further differentiation into elongating spermatids does not occur [73]. It is not yet clear whether retrotransposon methylation or expression is altered in *Miwi*$^{-/-}$ mice. Interestingly, piRNAs become more abundant, and contain a lower proportion of retrotransposon-derived sequences, during the pachytene spermatocyte-round spermatid stages of spermatogenesis when *Miwi* is expressed [70,71]. Miwi is a component of the chromatoid body, a

germ cell-specific cytoplasmic structure that appears to be involved in storing and processing RNAs [75]. Furthermore Miwi, Mili and piRNAs are all associated with the translational machinery in mouse germ cells [75,72]. Thus at least some aspects of piRNA/Piwi-like protein function in developing germ cells may be carried out by these complexes regulating gene expression at a post-transcriptional level.

To date the role of piRNAs in retrotransposon silencing has mainly been inferred from their physical association with the Piwi-like proteins as the large number of piRNA sequences in the genome does not make these sequences attractive targets for genetic deletion. However analysis of mice that carry a deletion of a piRNA cluster on chromosome 2 provides some direct evidence that piRNAs play a role in silencing retrotransposons in the germline [76]. Animals carrying a deletion of this piRNA cluster are fertile with no obvious developmental defects in gametogenesis but show a small ~1.6 fold increase in the abundance of LINE1 transcripts in their testes. However, LINE1 protein levels in these mutant testes are around 15-fold higher than in control testes, and the upregulation of LINE1 protein occurs specifically in meiotic spermatocytes [76]. Thus piRNA-mediated silencing of retrotransposons in male germ cells may involve post-transcriptional regulation of retrotransposon gene expression in meiotic spermatocytes in addition to de novo methylation of retrotransposon DNA in quiescent prospermatogonia.

Additional genes involved in silencing retrotransposons in the male germline

Some components of the chromatoid body have also been reported to play a role in suppression of retrotransposons during mouse spermatogenesis. Loss of the chromatoid body component Tdrd1 results in male infertility with some, but not all, spermatocytes failing to progress through meiosis [77]. Tdrd1 physically interacts with Mili and influences which piRNAs associate with Mili [78]. Loss of Tdrd1 results in increased expression of LINE1, but not IAP retrotransposons, in the testis and reduced DNA methylation of LINE1 retrotransposons in testicular germ cells [78]. Furthermore, mice carrying mutations in the chromatoid body component *Mael* exhibit defects in

silencing of both IAP and LINE1 retrotransposons in the germ cells [79]. *Mael*$^{-/-}$ spermatocytes initiate meiotic recombination and assemble the axial element of the synaptonemal complex, but homologous chromosome synapsis is impaired and the *Mael*$^{-/-}$ spermatocytes undergo apoptosis resulting in male infertility [79]. Interestingly, IAP and LINE1 elements are hypomethylated in *Mael*$^{-/-}$ testes suggesting that the *Mael*-dependent pathway for retrotransposon suppression may be linked to DNA methylation-mediated silencing of retrotransposons. However, in contrast to *Dnmt3L*$^{-/-}$, *Miwi2*$^{-/-}$ and *Mili*$^{-/-}$ mice, upregulation of retrotransposons is not seen in mitotic spermatogonia [61,62,79]. The molecular basis for this difference is not yet clear, but it is possible that the retrotransposon methylation that is established in quiescent prospermatogonia is not sufficient to silence retrotransposon expression later in meiosis, and that *Mael* is required to re-establish or maintain retrotransposon methylation before or during meiosis. However, although some components of the chromatoid body appear to be required for silencing of retrotransposons, other components of the chromatoid body are not required for this process [80], and the chromatoid body is likely to perform multiple functions in regulating gene expression in spermatogenesis [81].

Given the large number of different retrotransposon families in the mouse genome, it is possible that germ cells use multiple mechanisms to silence retrotransposons, and that different retrotransposon families have varying susceptibilities to different silencing mechanisms. One such silencing mechanism appears to depend on *Tex19.1*, a mammalian-specific gene of unknown biochemical function that is expressed in germ cells and pluripotent stem cells [82,83]. *Tex19.1*$^{-/-}$ knockout male mice are usually infertile and *Tex19.1*$^{-/-}$ knockout spermatocytes exhibit defects in progression through meiosis [83]. *Tex19.1*$^{-/-}$ knockout spermatocytes are able to initiate meiotic recombination and assemble the axial elements of the synaptonemal complex, but chromosome synapsis is impaired in many of these cells [83]. In *Tex19.1*$^{-/-}$ knockout testes, there is also an increase in abundance of transcripts from the MMERVK10C endogenous retrovirus, but not transcripts from the IAP endogenous retroviruses or LINE1 elements. Furthermore, the

MMERVK10C endogenous retrovirus is upregulated specifically in meiotic spermatocytes in *Tex19.1*$^{-/-}$ knockout mice, but not in mitotic spermatogonia [83]. Thus both retrotransposable element specificity and the timing of retrotransposon mis-expression differ between *Tex19.1*$^{-/-}$ mice and *Dnmt3L*$^{-/-}$, *Miwi2*$^{-/-}$ and *Mili*$^{-/-}$ mice. In addition there is no detectable change in the DNA methylation status of MMERVK10C retrotransposons in *Tex19.1*$^{-/-}$ knockout testes [83]. Thus *Tex19.1* does not appear to be involved in the *Dnmt3L*/*Mili*/*Miwi2*-dependent mechanism that methylates and silences retrotransposons in quiescent prospermatogonia. *Tex19.1* may play a role in post-transcriptional silencing of retrotransposons during spermatogenesis, although further work is needed to clarify the role of *Tex19.1* in retrotransposon silencing in the germline

Retrotransposon silencing in the female germline

DNA methylation has been proposed to be required for silencing retrotransposons and progression through meiosis in the female germline [60]. In contrast to male germ cells, de novo DNA methylation of retrotransposons in female germ cells does not require *Dnmt3L* and occurs in growing oocytes, i.e. after the oocytes have progressed through early meiotic prophase and completed chromosome synapsis [10,84]. However, the DNA methylation marks that are present in meiotic oocytes in the foetal ovary appear to be involved in silencing retrotransposons at this stage of development. Lsh is a chromatin remodelling protein that is required to maintain DNA methylation and silencing of retrotransposons in somatic cells [85]. In *Lsh*$^{-/-}$ female embryos, DNA methylation at IAP retrotransposons is also reduced in meiotic oocytes, and these oocytes are unable to progress through early meiotic prophase [60]. The *Lsh*$^{-/-}$ oocytes are able to initiate meiotic recombination and assemble the axial elements of the synaptonemal complex, but have defects in chromosome synapis [60]. The loss of DNA methylation at retrotransposon sequences and meiotic defects in *Lsh*$^{-/-}$ oocytes bears some resemblance to the loss of DNA methylation at retrotransposons and meiotic defects in *Dnmt3L*$^{-/-}$, *Miwi2*$^{-/-}$ and *Mili*$^{-/-}$ spermatocytes.

Although the Piwi-related proteins have an important role in silencing retrotransposons in the male germline, their role in silencing retrotransposons in the female germline is less clear. *Miwi*$^{-/-}$, *Mili*$^{-/-}$ and *Miwi2*$^{-/-}$ knockout female mice are all fertile, and expression of *Miwi* and *Miwi2* is mainly restricted to the male germ line [67,68,73]. However *Mili* is also expressed in the female germline [74] and there is a ~3.5-fold increase in the abundance of IAP retrotransposon transcripts in *Mili*$^{-/-}$ growing oocytes [86]. Thus *Mili* appears to be involved in silencing of retrotransposons in female germ cells, although the derepression of IAP retrotransposons that occurs in *Mili*$^{-/-}$ growing oocytes is not sufficient to impair fertility. It will be of interest to determine how *Mili* mediates repression of IAP retrotransposons in growing oocytes. Female germ cells appear to use endogenous siRNAs as well as piRNAs to constrain expression of transposable elements [86,87]. Interestingly, different retrotransposon sequences may be targeted by different silencing mechanisms during post-natal oocyte growth: whereas loss of *Mili* results in upregulation of IAP elements, loss of the Dicer-mediated siRNA pathway results in upregulation of SINE elements and the RLRT10, MT and MTA endogenous retroviruses [86,87]. It is not yet clear whether these silencing mechanisms feed-back to influence methylation of retrotransposon DNA in the female germline in mice, or whether these silencing mechanisms also operate during earlier stages of female germ cell development when the germ cells are progressing through early meiotic prophase.

A role for retrotransposon silencing in mouse meiosis?

Many of the mouse mutants described above, such as *Dnmt3L*$^{-/-}$, *Miwi2*$^{-/-}$, *Mael*$^{-/-}$ and *Tex19.1*$^{-/-}$ that exhibit defects in retrotransposon silencing also have defects in progression through meiosis. Defects in retrotransposon silencing do not appear to be a general consequence of defective chromosome synapsis during meiosis [68], however it is not clear if defects in retrotransposon silencing cause the defects in meiosis that are seen in each of these mouse mutants, or whether these two events are merely indirectly associated. As much of the evidence for an association between retrotransposon silencing and progression through meiosis is genetic, the possibility that some aspects of these mutant mouse phenotypes are indirect and independent consequences of subtle changes in gene expression cannot be excluded. Furthermore it is also possible that some of these mouse mutants are affecting global chromosome structure, and that defects in global chromosome structure during meiosis might independently cause de-repression of retrotransposons and aberrant chromosome synapsis. Alternatively the association between defective retrotransposon silencing and aberrant progression through meiosis in each of these mouse mutants may be indicative of a direct causal link between these events.

There appears to be some similarity between the meiotic defects in *Dnmt3L*$^{-/-}$, *Mael*$^{-/-}$ and *Tex19.1*$^{-/-}$ spermatocytes [64,79,83]. In each of these mutants, early markers of meiotic recombination such as γH2AX, Dmc1 and Rad51 are present on the meiotic chromosomes, and axial elements of the synaptonemal complex assemble. In *Dnmt3L*$^{-/-}$, *Mael*$^{-/-}$ and *Tex19.1*$^{-/-}$ knockout mice, the extent of chromosome synapsis varies between spermatocytes, and between chromosomes within each spermatocyte, and it is not clear whether synapsis or asynapsis is occurring preferentially on certain homologous chromosomes. As might be expected given the relationship between synapsis and recombination, the recombination sites appear to mature in the regions where synapsis has occurred, but not in the regions of asynapsis. In *Mael*$^{-/-}$ knockout spermatocytes, homologous chromosome pairing appears to have occurred between some asynapsed chromosomes but not others [79]. There

appears to be little evidence of homolog pairing between asynapsed chromosomes in *Tex19.1*$^{-/-}$ and *Dnmt3L*$^{-/-}$ spermatocytes [64,83]. The meiotic defects in these mutants are consistent with defects in some aspect of meiotic recombination, homolog pairing or the initiation or progression of synapsis between homologous chromosomes, but the interdependence of these meiotic events makes it difficult to distinguish the nature of the primary defect.

One possible reason why de-repression of retrotransposons might cause defects in meiotic progression is that increased retrotransposition may cause DNA damage that interferes with the normal double-strand DNA break-dependent process of meiotic recombination, or activates checkpoint systems. The presence of extensive retrotransposition-induced DNA damage in *Mael*$^{-/-}$ meiotic spermatocytes has been elegantly shown by crossing *Mael*$^{-/-}$ mice with *Spo11*$^{-/-}$ mice to allow retrotransposition-induced DNA double-strand breaks to be detected in the absence of confounding meiotic *Spo11*-dependent DNA double strands breaks [79]. Excessive retrotransposition-induced DNA damage could perturb meiotic recombination by sequestering meiotic recombination proteins, or by disrupting meiotic recombination intermediates, resulting in secondary defects in homolog pairing or synapsis. There are some mouse lines that exhibit increased levels of retrotransposon expression in spermatocytes without disrupting progression through meiosis [76,88]; however the level and developmental timing of the increase in retrotransposon expression in these mice may not be sufficient to disrupt meiotic recombination.

Similarly it is also possible that increased expression of retrotransposon-encoded proteins, rather than active retrotransposition, induces cytotoxicity in germ cells due to interacts with endogenous germ cell proteins and/or cellular machinery. In this scenario the developmental timing of the spermatogenic arrest in *Dnmt3L*$^{-/-}$, *Miwi2*$^{-/-}$, *Mael*$^{-/-}$ and *Tex19.1*$^{-/-}$ may be more related to the tendency of retrotransposons to be expressed during meiotic prophase [89] than any specific interaction between the retrotransposition machinery and meiotic recombination. Indeed in

Dnmt3L$^{-/-}$ and *Miwi2*$^{-/-}$ mutant mice where retrotransposons are de-repressed in spermatogonial stem cells and spermatogonia as well as meiotic spermatocytes, there appear to be some defects in the spermatogonia and/or spermatogonial stem cells as well in the meiotic spermatocytes [61,68,90]. Furthermore, as expression of retroviral *Env* proteins can be sufficient to induce apoptosis in somatic cells in culture [91], and ectopic expression of the human endogenous retrovirus-encoded *Rec* gene in transgenic mice is sufficient to disrupt spermatogenesis [92], the possibility that cytotoxicity of individual retrotransposon-encoded proteins may contribute to germ cell apoptosis in *Dnmt3L*$^{-/-}$, *Miwi2*$^{-/-}$, *Mael*$^{-/-}$ and *Tex19.1*$^{-/-}$ mutant mice cannot be excluded.

An alternative possibility is that silencing of retrotransposons may be important to prevent retrotransposon sequences from interfering with the homology search during chromosome pairing. As retrotransposons are abundant repetitive elements distributed throughout the mammalian genome, the interactions between these sequences could potentially facilitate pairing between non-homologous chromosomes during meiosis. Indeed, retrotransposon sequences tend to be associated with chromosomal axes rather than loops in meiotic chromosomes which may help prevent these repetitive sequences interfering with the homology search [20]. In some organisms the presence of transcriptionally active or repressive chromatin structures appears to influence the frequency of meiotic recombination at some genomic loci [93,94]. If analogous mechanisms also operate in mice then derepression of retrotransposons may allow these repetitive sequences to participate in the homology search causing some non-homologous pairing, or the sequestration of some early recombination sites into sequences that are not productive for chromosome pairing. This mechanism could explain the meiotic defects in *Dnmt3L*$^{-/-}$ and *Mael*$^{-/-}$ knockout mice where there appears to be an effect on transcriptional silencing of highly abundant LINE1 and IAP retrotransposons. However it is less clear whether *Tex19.1*$^{-/-}$ knockout mice disrupt transcriptional repression of retrotransposons, or whether the retrotransposons sequences affected in *Tex19.1*$^{-/-}$ mice are sufficiently abundant to induce this type of effect.

Further work should help to elucidate the molecular basis of the association between retrotransposon silencing and progression through meiosis, and may also help our understanding of some of the fundamental chromosomal events that occur during germ cell development in mice. It is not yet clear how germ cells distinguish retrotransposon sequences from endogenous genes in the genome, or how chromosomes in meiotic germ cells distinguish their homologs from the other chromosomes that are present in the nucleus. However germ cells must be able to balance the opposing demands of limiting the frequency of potentially mutagenic events and creating sufficient genetic variation during meiosis to achieve evolutionarily viable rates of genetic and chromosomal stability as genetic information is passed from one generation to the next.

Figure Legends

Figure 1. The mammalian germline cycle.

A male sperm (blue) and a female egg (pink) fuse together at fertilization to produce a pluripotent zygote. The pluripotent cells (orange) present in early development will in turn giver rise to germ cells (green) later in development that will undergo meiosis to reduce their chromosome content while differentiating into either male sperm of female eggs to give rise to the next generation.

Figure 2. Germ cell development in mice.

Pluripotent cells (orange) are present in mouse embryos early in development. By 7.5 days post coitum (dpc) germ cells (green) have differentiated from the pluripotent cells. The germ cells proliferate and migrate to the emerging gonads, and are scattered throughout the developing gonad by 11.5 dpc. In male embryos, the germ cells enter quiescence and become arranged within testis cords. A few days after birth some of the male germ cells differentiate into spermatogonial stem cells, which provide a continuous supply of spermatogonia that undergo meiosis and differentiate into mature sperm in the adult mouse. In female embryos the germ cells initiate meiosis at around 13.5 dpc, but progression through meiosis arrests a few days after birth. Meiosis resumes after the oocytes are fully grown in adult mice, but is not completed until fertilization.

Figure 3. Chromosomal behaviour during mouse meiosis.

Post-mitotic/premeiotic germ cells contain two homologous copies of each chromosome (orange and brown threads, only two homologous chromosome axes are shown for clarity). During the preleptotene stage of meiosis (not shown) DNA replication duplicates each chromosome, and the meiosis-specific cohesions (black rings) start to assemble to help maintain the connection between the resulting sister chromatids. In leptotene the condensing chromosomes are visible as discrete fine threads by light microscopy, meiotic recombination is initiated (not shown) and the axial elements of the synaptonemal complex assemble along the chromosomal axes (not shown). During zygotene, homologous chromosomes start to pair then synapse as the synaptonemal complex continues to

assemble (black zipper-like structure). In pachytene the homologous chromosomes are completely synapsed and recombination between homologous chromosomes is completed to generate crossover sites. During diplotene, the synaptonemal complex disassembles and the crossover sites mature into chiasmata that act as a physical connection between homologous chromosomes. The chromosomes condense fully in diakinesis (not shown) and the nuclear envelope disintegrates. During metaphase I the homologous chromosomes align on the meiotic spindle, and cleavage of cohesin subunits along the chromosome arms allows the chiasmata to resolve and homologous chromosomes to segregate during anaphase I. Cleavage of centromeric cohesins in meiosis II then allows sister chromatids to segregate. Each round of meiosis generates four genetically distinct haploid products from a diploid cell.

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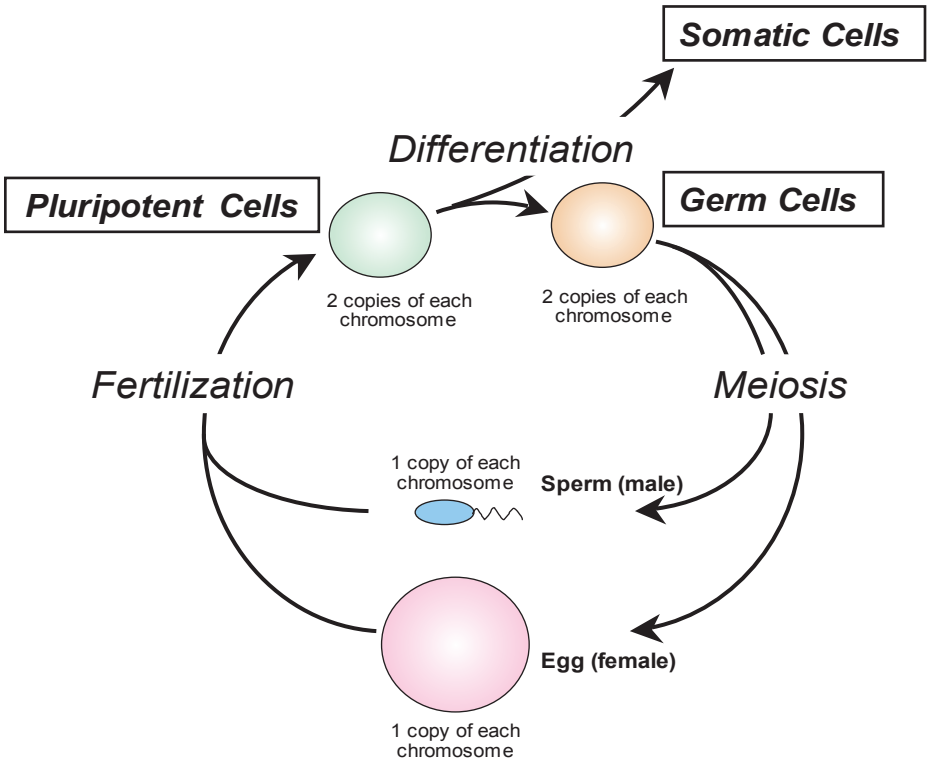
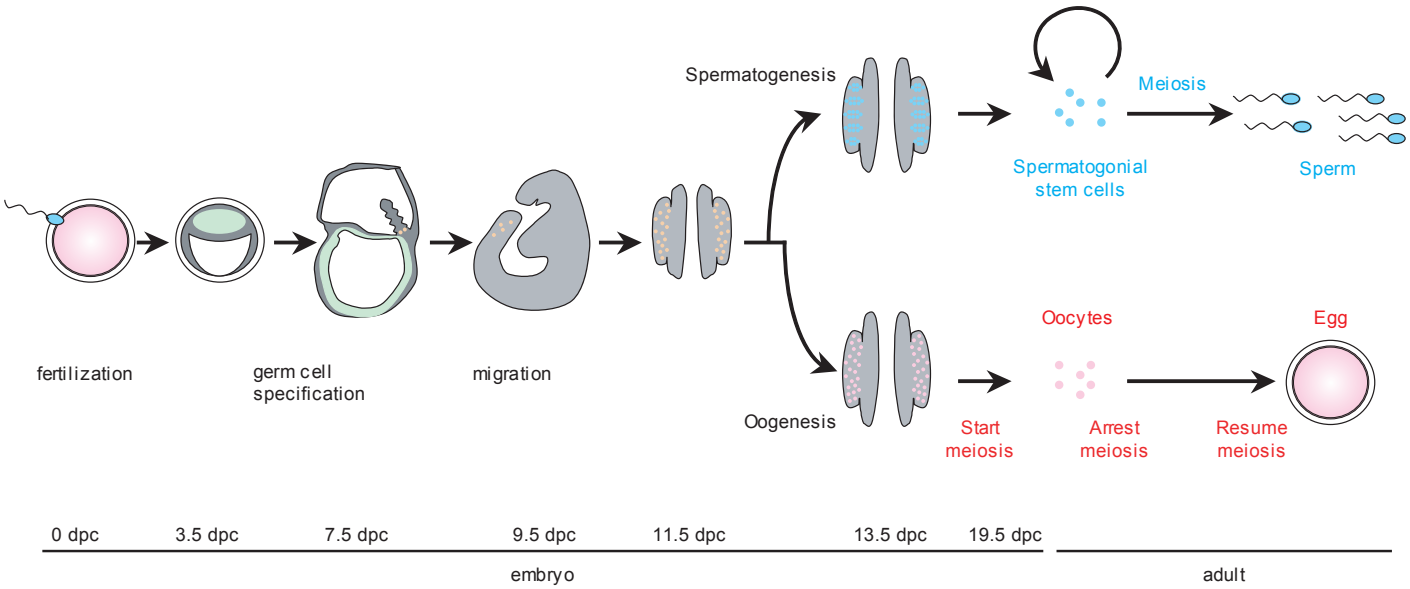

Somatic Cells
Differentiation
Pluripotent Cells
Germ Cells
2 copies of each chromosome
2 copies of each chromosome
Fertilization
Meiosis
1 copy of each chromosome
Sperm (male)
Egg (female)
1 copy of each chromosome

Figure 1


Spermatogenesis
Meiosis
Spermatogonial stem cells
Sperm
fertilization
germ cell specification
migration
Oogenesis
Oocytes
Egg
Start meiosis
Arrest meiosis
Resume meiosis
0 dpc
3.5 dpc
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9.5 dpc
11.5 dpc
13.5 dpc
19.5 dpc
embryo
adult

Figure 2

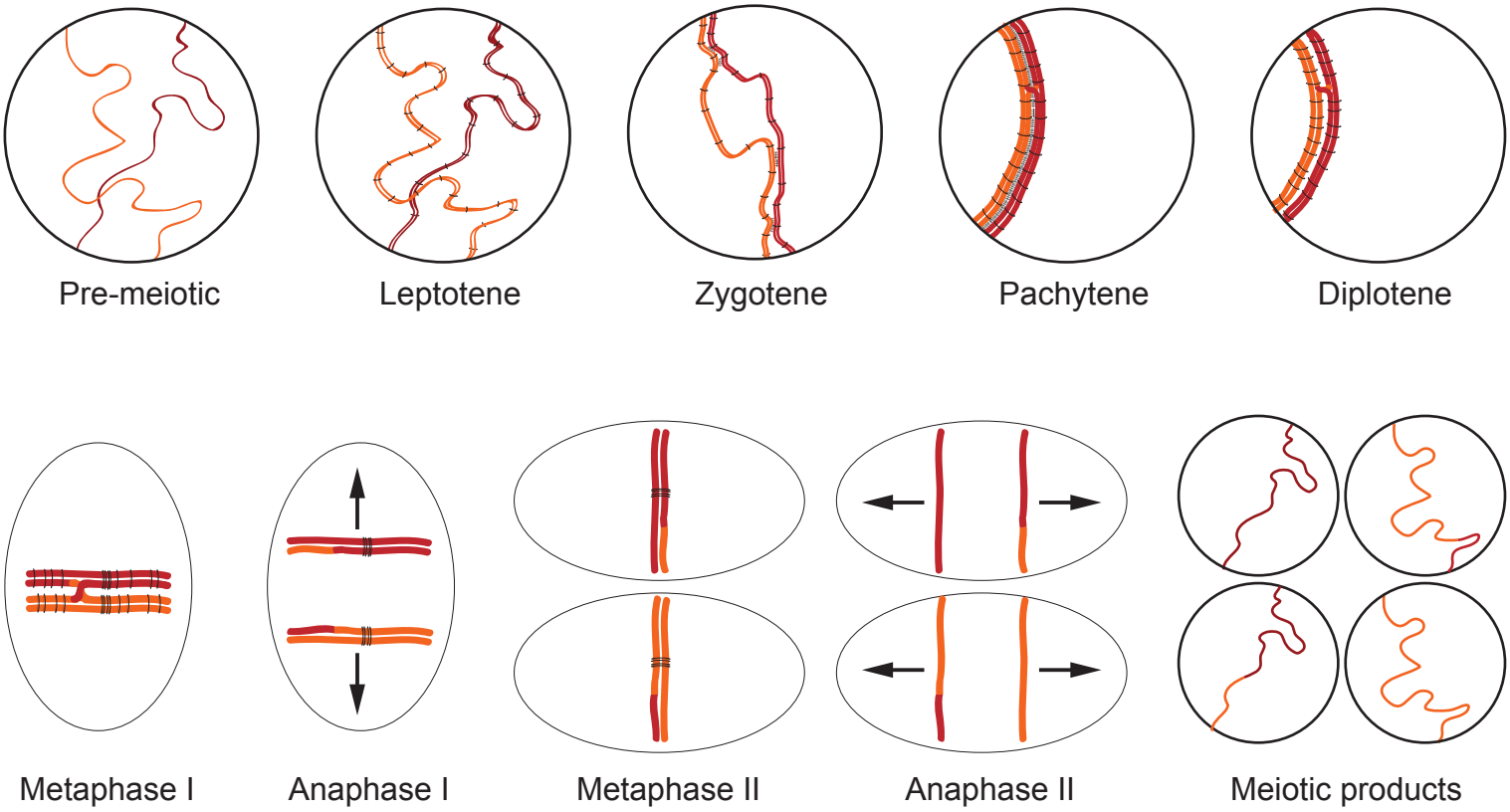
Pre-meiotic
Leptotene
Zygotene
Pachytene
Diplotene
Metaphase I
Anaphase I
Metaphase II
Anaphase II
Meiotic products

Figure 3