

Review Article

Meiosis initiation: a story of two sexes in all creatures great and small

Ieng Fong Sou^{1,2}, Rebecca M. Pryce¹, Wee-Wei Tee^{2,3} and  Urszula Lucja McClurg¹

¹Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool L69 7ZB, U.K.; ²Chromatin Dynamics and Disease Epigenetics Group, Institute of Molecular and Cell Biology, Agency for Science, Technology and Research (A*STAR), Singapore, Singapore; ³Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

Correspondence: Urszula Lucja McClurg (Urszula.McClurg@liverpool.ac.uk) or Wee-Wei Tee (wwtee@imcb.a-star.edu.sg)



Meiosis facilitates diversity across individuals and serves as a major driver of evolution. However, understanding how meiosis begins is complicated by fundamental differences that exist between sexes and species. Fundamental meiotic research is further hampered by a current lack of human meiotic cells lines. Consequently, much of what we know relies on data from model organisms. However, contextualising findings from yeast, worms, flies and mice can be challenging, due to marked differences in both nomenclature and the relative timing of meiosis. In this review, we set out to combine current knowledge of signalling and transcriptional pathways that control meiosis initiation across the sexes in a variety of organisms. Furthermore, we highlight the emerging links between meiosis initiation and oncogenesis, which might explain the frequent re-expression of normally silent meiotic genes in a variety of human cancers.

Meiosis in single cell organisms

Ultimately, the decision to enter into meiosis is vital for the creation of variance within species. therefore, meiotic entry is tightly regulated. Although fundamental characteristics of meiosis, such as the mechanics of homologous recombination, are highly conserved between species the decisive signals required to enter meiosis, and its regulation, are divergent in a variety of organisms.

Single celled eukaryotes generally undergo meiosis only when ‘pressured’ to do so, with meiotic entry in both budding and fission yeast governed by relative nutritional availability. When growth conditions are optimal, mitosis is the default division process for single celled organisms since it enables rapid growth utilising the available nutrients. However, in response to nutrient-restriction, yeast signalling mechanisms lead to activation of meiotic programmes that have the ability to generate progeny that possess increased resilience to the newly applied pressure (Figure 1) [1]. Consequently, high concentrations of nitrogen and glucose can be considered inhibitors of budding yeast meiosis, which makes *Saccharomyces cerevisiae* one of the simplest organisms among laboratory models of meiosis. Budding yeast exist as both haploid and diploid with haploid yeast characterised by ‘a’ or ‘ α ’ mating type (MAT), mating produces a diploid (MATa/ α) [2]. In response to nitrogen starvation or lack of fermentable carbon sources, diploid mitotic cells arrest at the G1 phase of mitosis and prepare for meiotic entry through global transcriptional activation of meiosis-associated genes [3,4]. Removal of nitrogen and glucose induces expression of Inducer of MEiosis 1 (IME1), a protein kinase master regulator of core meiotic proteins such as DMC1 and REC8 [1,5], which is repressed by Repressor of MEiosis 1 (RME1) in haploid cells. Consequently, haploid cells are not capable of responding to environmental cues and only MATa/ α diploid cells can undergo meiosis as the complex formed by a1- α 2 is able to repress expression of RME1 [6–8]. Nutritional regulation of IME1 expression in diploid cells is executed by Protein Kinase A (PKA) and Target Of Rapamycin Complex 1 (TORC1). In the presence of glucose, when cAMP levels are high, the PKA pathway is activated by the G-protein α subunit GPA2 allowing it to phosphorylate SOK2, which in turn acts to repress IME1 expression.

Received: 2 June 2021
Revised: 29 September 2021
Accepted: 6 October 2021

Version of Record published:
28 October 2021

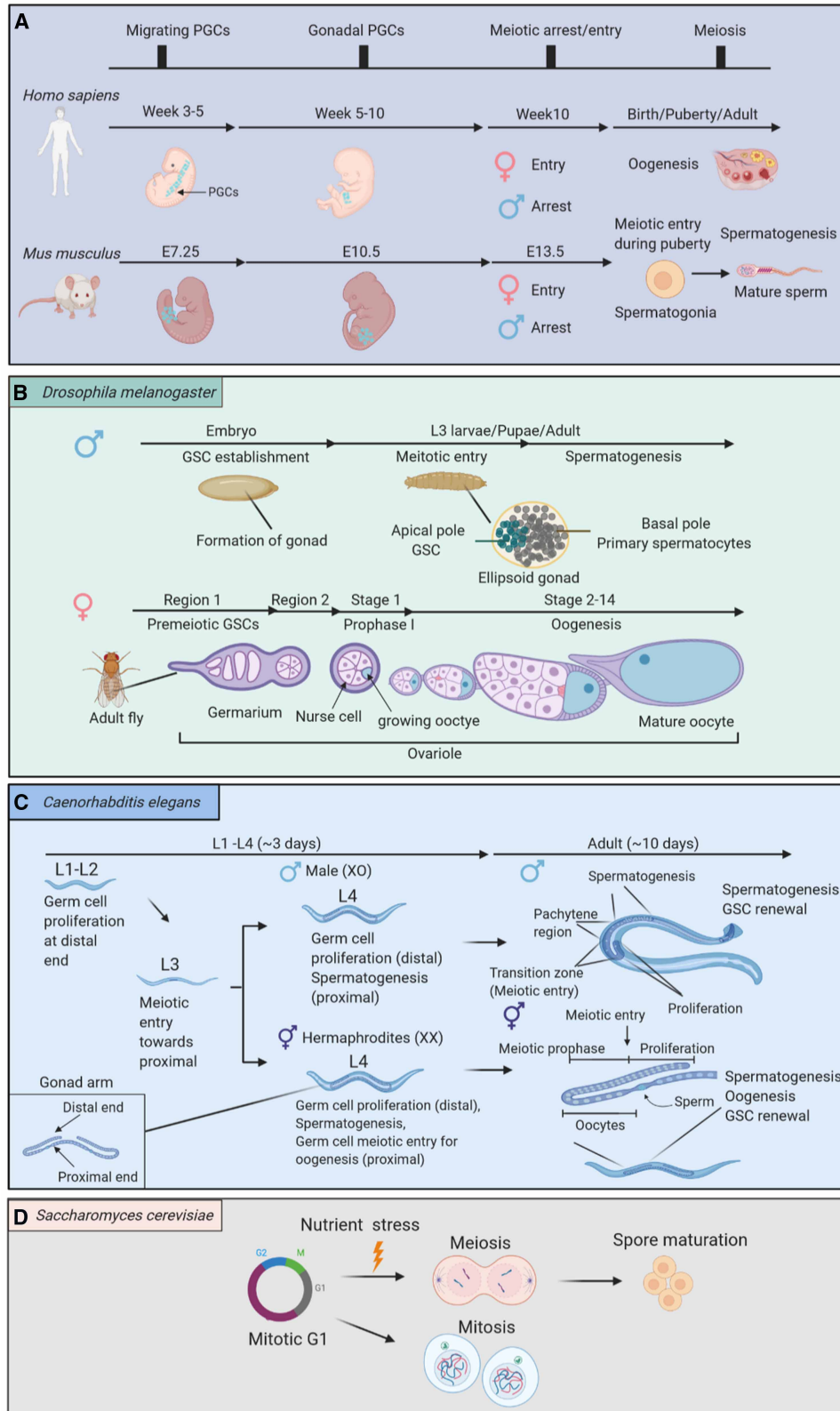


Figure 1. The timing of meiotic entry in distinct species and sexes.

Part 1 of 2

(A) In *Homo sapiens* and *Mus musculus*. Meiotic entry is largely conserved. In general, PGCs migrate through the midgut and arrive in the fetal gonads, where meiosis occurs in females and mitosis arrests in males. (B) In *Drosophila melanogaster* germ cells

Figure 1. The timing of meiotic entry in distinct species and sexes.

Part 2 of 2

are derived from GSCs. Meiosis occurs in L3 larval instar stage in the male, GSCs shift from the apical pole to differentiate into primary spermatocytes in the basal pole, whereas GSCs enter prophase I within region 2 of germarium in adult female ovariole. (C) In *Caenorhabditis elegans* the dioecious hermaphroditic and male worms both enter meiosis during the L3 stage, GSCs shift from the distal to the proximal end as meiosis progresses. During L4 stage, male worms undergo spermatogenesis similarly to hermaphroditic worms but in hermaphrodites a subgroup of germ cells enter meiosis for oogenesis. With the renewal of GSCs, oogenesis and spermatogenesis can be maintained throughout adulthood. (D) *Saccharomyces cerevisiae* reproduces asexually upon nutrient starvation mitosis arrests at G1 and meiotic entry is triggered.

Furthermore, activation of TORC1 through nitrogen availability sensing, also represses IME1 [9]. Upon nutrient withdrawal, reduced activity of the PKA pathway allows for IME1 expression and increased activity of the RIM11 kinase. RIM11 phosphorylates UME6 and IME1, which are then able to form a stable complex leading to the expression of early meiotic genes including the serine/threonine kinase Inducer of MEiosis 2 (IME2) [10]. This provides a negative feedback loop allowing cells to control meiotic timing as IME2 can lead to IME1 proteasomal degradation [11]. This flexible phosphorylation-dependent meiosis-mitosis switching mechanism provides yeast with survival and growth advantages, by ensuring usage of available nutrients and generation of new gametes which might gain the ability to survive 'harsh' environments [12].

Interestingly, as a consequence of adapting to different growth environments fission yeast appear to lack an IME1 orthologue. Meiotic initiation is instead governed by STE11⁺ with expression controlled by down-regulation of TORC1 and PKA signalling, similarly to IME1 in budding yeast. However, the main driver of meiosis initiation is not lack of glucose but rather nitrogen starvation [13]. Nitrogen limitation activates expression of STM1⁺, a GPA2⁺ inhibitor. Once expressed STM1⁺ leads to STE11⁺ expression regardless of glucose levels [14]. Further up-regulation of STE11 after conjugation occurs as diploids deactivate the PAT1 kinase through a decrease in phosphorylation [15]. This facilitates the activation of early meiotic genes such as MEI2⁺, PAT1 down-regulation is critical for MEI2⁺ activity. When growth conditions are optimal PAT1 phosphorylates MEI2 and STE11 leading to proteasomal degradation of MEI2 and STE11Rad24 binding which inhibits STE11 transcription factor activity.

The timing of meiotic onset in multicellular organisms

The nutrient restriction requirement for meiosis initiation in *S. cerevisiae* and *S. pombe* is well characterised and readily replicated within the laboratory, making yeast a useful tool in meiotic research. However, multicellular organisms have an added layer of complexity as meiosis cannot be activated synchronistically in every cell of the organism and as such a complex system of regionalising meiosis, and consequently meiosis initiation, has developed. Consequently, in multicellular organisms specialised germline stem cells (GSC), which arise during embryogenesis, can divide to renew the GSC pool and differentiate into sex-specific germ cells that undergo meiosis [16]. However, the timing of meiotic initiation, as well as transcriptional and translational changes during meiosis, differ significantly between the sexes. In this section, we will discuss how organisation of meiosis initiation differs between the common multicellular models of meiosis by comparing and contrasting the context of meiosis initiation, its timing and germ cell origins.

Due to its regionalised nature, meiosis initiation in multicellular organisms depends on the migration of germ cells, highlighting the need for evolutionarily conserved mechanisms to facilitate germ cell development. In *Drosophila melanogaster* males and females exist as separate organisms and future gametes arise from GSCs in the testis and ovaries, respectively. These GSCs are differentiated from a subgroup of Primordial Germ Cells (PGCs) during the embryonic stage [17,18]. In contrast with humans, meiosis initiation in the fruit fly occurs much earlier in males than in females (Figure 1). In male flies, entry into meiosis and spermatogenesis is established in L3 larval stage within the ellipsoid gonad, where GSCs reside in the apical pole and differentiate into the primary spermatocytes within the basal pole [19]. At the apical tip of the testis resident hub cells divide mitotically and are crucial for GSC maintenance. Each GSC is surrounded by two cyst stem cells. GSCs divide asymmetrically to sustain the GSC pool and produce a gonialblast cell (GB). The GB cell remains associated with the cyst cells. GB divides mitotically, driven by Bam signalling, as in females [18,20]. Bam levels peak at the 8-cell M stage and these 8 cells enter their final division before meiotic onset [21]. The 16-cell cyst containing

spermatogonia undergoes a final division to produce 64 spermatids. Mature gamete production continues throughout adulthood as males maintain a nice of 6–12 germ stem cells allowing testis homeostasis [19,22].

In female *Drosophila*, the GSC niche is established in L3 larval stage within the female gonad [23]. Meiosis occurs within the adult germarium located at the tip of the adult ovariole, which consists of three regions [24]. When a GSC divides, one of the daughter cells is driven further away from the cap cells due to spatial restriction. This cell is known as a cytoblast. In *Drosophila* proximity to bone morphogenic protein (BMP) signalling prevents cell differentiation through repression of Bam (bag of marbles), similarly to distal tip cell signalling that prevents meiotic entry in *C. elegans*. Once Bam is no longer repressed by BMP expression in the cytoblast (CB), it becomes a major driver of GSC differentiation [25]. Differentiated cyto blasts are able to divide mitotically until Bam levels peak before the final mitotic division producing a 16-cell cyst, of which just a single cell will progress through meiosis to become an oocyte. Whereas Bam is necessary in female flies for initial GSC differentiation as well as CB divisions, it is needed only for subsequent gonialblast division in males. In contrast with mammals, *Drosophila* females maintain a 2–3 GSC niche throughout adulthood giving them the ability to produce oocytes throughout their lifetime. Fly germ cell development is similar to mammals, since it is supported by surrounding somatic cells to provide essential cellular signalling molecules for germ cell maturation, leading to downstream transcriptional activation and subsequent embryonic development.

In contrast with *Drosophila*, the multicellular model worm *Caenorhabditis elegans* has the ability to produce both types of gamete within a single organism. Furthermore, the gonads are easily visible due to a transparent body, and therefore cytological analysis is possible on the whole organism, as well as on a single cell level. Experimentally manipulatable meiotic cells are in great supply with 50% of the total nuclei belonging to the germline [26], making *C. elegans* an incredibly useful tool for meiotic research.

Germline sex determination in *C. elegans* is controlled by a network of >20 genes. *C. elegans* possess both male and female reproductive organs. However, they can still produce male worms (designated X0) with a single X chromosome originating from the male germ cells, although this occurs at a very low frequency (<0.2% of total). The male phenotype results from the mis-segregation of X chromosomes in oocytes which can lead to oocytes with no X chromosome, which are then fertilised by sperm [27]. However, the vast majority (99.8%) of *C. elegans* are hermaphrodite (XX) worms which can self-fertilise and are able to perform both oogenesis and spermatogenesis [27]. In *C. elegans*, similarly to *Drosophila*, mitosis-to-meiosis transition is determined in a spatiotemporal manner. In *C. elegans* meioocytes progress through meiosis as they move away from the distal tip of the gonad. Germline stem cells are located at the distal gonad tip, surrounded by the distal tip cell (DTC). The DTC is adjacent to a region known as the progenitor zone, which contains a mixed population of mitotically dividing cells organised into population pools, beginning with GSC, followed by mitotically dividing cells and ending with meiotic S phase cells [28]. At least three complementary signalling pathways promote meiotic entry in worms: the RNA-binding protein GLD-1 (Germline Defective-1), GLD-2, and the recently discovered SCFPROM1 ubiquitin ligase complex. GLD-1/Notch signalling acts to inhibit the translation of mitotic cell cycle proteins, whereas the GLD-2 pathway leads to the translational activation of meiotic entry proteins. The SCFPROM-1 complex promotes degradation of mitotic cell cycle proteins [29]. The DTC releases GLP-1 activating ligands which promote proliferation of GSC and inhibit both GLD-1 and GLD-2 pathways repressing meiotic entry [30]. In *C. elegans*, germ cell amplification within the distal end is caused by interactions with sheath cells (sh1) during the L1–L2 stage until elongation of the gonad; consequently germ cells migrate further away from the distal end and are no longer controlled by GLD-1 signalling, which leads to meiotic entry [31]. Following the loss of GLP-1 (abnormal Germ Line Proliferation 1) signalling, mitotic cells complete their final division and enter meiosis, suggesting that GLP-1 is acting as a repressor of meiosis [32]. Thus, meiotic entry in *C. elegans* is controlled by spatiotemporal movement away from GSCs and the change in external signalling is associated with this movement. To ensure that hermaphrodites can self-fertilise, entry into male meiotic prophase I occurs in late L3 stage and mature sperm can be observed during L4 stage. However, germ cells that enter meiosis from mid-L4 onwards are differentiated into oocytes throughout adulthood [33]. In rare male worms, germ cells enter pachytene during mid L3 and undergo spermatogenesis from mid L4 and throughout adulthood (Figure 1) [33].

Regulation of female meiosis in *Xenopus laevis*

One of the major difficulties associated with meiotic research lies in appreciating the differential regulation between male and female meiosis. Vertebrate female meiosis presents the additional challenges of prolonged duration, ethical issues and limited access to experimental material. The *Xenopus laevis* model system has been

instrumental in studying the later stages of female meiosis due, in part, to the large size of the mature egg (~1 mm diameter) and an ability to manipulate its contents via both immunodepletion and microinjection of experimental biomolecules and the generation of cell-free nuclear extracts, *ex vivo* [34]. The latter is possible because eggs contain a reservoir of RNA and proteins allowing for 12 rounds of transcription-free cell division following fertilisation prior to the onset of zygotic transcription after the Mid-Blastula transition (MBT). These early divisions are driven by cyclical translation and degradation of cyclin B, consequently the *Xenopus* system was critical for the purification of the biochemical drivers of meiotic and mitotic cell cycles [35]. During oocyte development, cells are synchronously arrested in the first meiotic metaphase until meiotic maturation is triggered by progesterone, which can be recapitulated *ex vivo* after manual oocyte dissection from the ovary or after egg laying is induced with hCG (the origin of the use of *Xenopus laevis* for the detection of human pregnancy; the ‘Hogben’ Test). Meiotic cell cycle arrest is facilitated by high endogenous cAMP levels and a decrease in cAMP induces inhibition of the cAMP dependent protein kinase (PKAc), which is necessary for ending meiotic arrest [36] through modulation of the PKA substrate ARR19 [37], a key regulator of PP2A, which also has critical roles in later mitotic cell cycles [38]. Following completion of meiosis I, germinal vesicle breakdown (GVBD) proceeds the extrusion of the first polar body after which oocyte enters meiosis II without an intervening interphase and arrests at metaphase of the second meiotic division until fertilisation. MPF (maturation promoting factor), which is composed of cdc2 and B-type cyclins, is also critical for the G2-M transition [35,39] MPF activity is initiated by Mos, which activates the classical MAPK pathway, after which it is regulated in a cell cycle dependent manner by the CSF (cytostatic factor) [40]. Due to historical advantages of *Xenopus laevis* for developmental biology research [41], work has focused on the later stages of oocyte meiotic progression rather than early meiosis initiation, and will not be discussed further here. The *Xenopus* system and cell cycle are extensively reviewed in [34,42,43].

Spermatogenesis and oogenesis in mammals

Male and female gametes are distinct in size, shape and their relative contribution to the fertilised embryo. Cytoplasmic and nuclear contents in the sperm are reduced as the oocytes contribute to all the essential organelles for the future zygote and are metabolically active, whereas sperm cells contribute to the future zygote by delivering the paternal genome, two centrioles, oocyte activation components and transcripts essential for embryonic development [44,45]. Therefore, optimal (reduced) size and innate motility have been prioritised in order to reach the egg. Consequently, the oocyte requires a longer maturation period compared with the process of spermatogenesis. During oogenesis, the centrosomes are eliminated [46] and centrioles within the future zygote are provided by mature spermatids [47]. Therefore, meiosis is regulated differently in the context of meiotic entry, timing, hormonal changes and outcomes during oogenesis and spermatogenesis in order to produce high quality gametes that have contrasting roles prior to fertilisation.

In mammals, meiosis takes place after the migration of primordial germ cells (PGCs) to the developing gonads, followed by differentiating into oogonia and spermatogonia, which are the precursors of egg and sperm, respectively. PGCs differentiate into mature gametes in a specific organ, spermatogenesis in male testis and oogenesis in female ovaries. In humans, PGCs travel to the developing gonads through the midgut during week 3 to 5 of embryogenesis. Female oocytes enter meiosis at week 10 during fetal development, while male germ cells undergo mitotic G1 arrest until puberty, when meiosis is initiated (Figure 1) [48]. Regulation of meiotic entry and progression in mice is very similar to that in humans. In mice, PGCs migrate to the fetal gonad at embryonic stage E10.5 and enter meiotic prophase I in female when male germ cells commit to mitotic arrest at E13.5 (Figure 1) [49]. Similarly, to lower class species such as worms and flies, mammalian germ cells also require sex-specific somatic cells to support different stages of germ cell development.

Male meiosis is initiated by major male sex hormones such as pituitary-secreted follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone, which surge upon puberty [50]. In the testis, somatic supporting cells such as Sertoli and Leydig cells regulate spermatogenesis in an endocrine manner [50]. FSH acts on the Sertoli cell receptors while LH stimulates Leydig cells to secrete testosterone [51]. In concert with testosterone, FSH plays roles in providing essential factors and nutrients for male germ cell maturation [50]. These signals are required for spermatogonia to differentiate into primary spermatocytes allowing division into haploid secondary spermatocytes during meiosis I. Finally, spermatocytes differentiate into mature haploid spermatids within the seminiferous tubules of the adult testis [52]. To produce mature gametes throughout life, a proportion of spermatogonial stem cells (SSCs) can be renewed and differentiate, providing a continuous supply of sperm cells [53]. In spermatocytes, transcription is robustly activated from pre-meiotic (spermatogonia)

until post-meiotic (round spermatid) stages [54]. At the final stage of male meiosis, a wave of transcriptional activation is required for round spermatids to prepare for the dramatic morphological change into mature sperm, including replacement of histones with protamines, flagellar formation and cytoplasmic removal [55]. These features are required to reshape the nucleus, resulting in dramatic reduction in the nuclear volume and a complete cessation of transcriptional activities during nuclear compaction [55,56]. Consequently, the final stages of spermatogenesis are characterised by condensed chromatin and depend on stored paternal mRNAs to generate fertilization-competent spermatocytes [55].

Female PGCs differentiate into oogonia after arriving in the genital ridge and enter primary oocytes

In contrast with males, the number of female germ cells is limited with meiosis paused at different stages. Oocyte dictyate arrest in prophase I and oocyte maturation is supported by a group of somatic cells within the follicle known as granulosa cells, which wrap around the oocyte and provide a favourable environment to regulate maturation processes including growth, meiotic pausing and resumption. Female meiosis initiates in fetal ovaries followed by arrest at diplotene stage of prophase I caused by granulosa-secreted cyclic GMP (cGMP), propagated into the oocytes through gap junctions. During puberty, LH from the pituitary gland binds to the receptors on the granulosa cells resulting in a dramatic reduction in cGMP within the oocyte, which in turn resumes meiosis, resembling the GPCR-coupled nutrient starvation response present in yeast [57]. LH stimulation also leads to the breakdown of an oocyte-specific nuclear membrane called the germinal vesicle (GV), which marks the resumption of prophase I [58]. Oocyte maturation is facilitated by a group of cyclin-dependent kinases and cyclins including CDK1, CCNB1 and CCNB2 within the maturation promoting factor (MPF) complex [59]. At the end of meiosis I, homologous chromosomes are segregated into the secondary oocyte that proceeds into meiosis II and the first polar body, a small meiotically incompetent oocyte. At metaphase II, another arrest occurs in the secondary oocyte. This arrest is maintained by the cytostatic factor, which stabilises MPF activity and prevents cyclin B degradation induced by the elevation of Ca^{2+} levels when fertilisation occurs by fusing with the sperm [60]. Fertilisation terminates meiosis II, resulting in the protrusion of the second polar body and the mature fertilised ovum (now known as the zygote), which gives rise to the next generation [58]. Developing oocytes begin to acquire meiotic competence and become transcriptionally inactive until zygotic gene activation (ZGA) [61,62]. Oocyte maturation relies on stored dormant maternal transcripts that are generated during the primordial phase and translational control of these mRNAs is hence pivotal for oocyte maturation and early embryonic development [63].

Regulation of mammalian meiotic initiation

Meiotic entry is governed by numerous, intricate signalling mechanisms, a few of which are discussed above. The essential signals that propagate from the embryonic gonad to the PGCs for promoting gametogenesis have been exclusively reviewed by [48] and [64]. In mammals, typically, FSH stimulates meiotic entry and induces the production of all trans retinoic acid (RA) to facilitate germ cell differentiation. RA is a vitamin A derivative that binds to nuclear RA receptors (RAR) and retinoid X receptors (RXR), activating transcription of RAR and RXR target genes. Consequently, RA has historically been considered to be the master regulator of meiotic entry in both oogenesis and spermatogenesis, although the precise signalling mechanism may be species-dependent [65]. A body of published studies demonstrates that RA driven activation of STimulated by Retinoic Acid gene 8 (*Stras8*) is crucial for meiotic onset [66–69]. Although STRA8 is normally cytoplasmic, it localises to the nucleus during meiotic onset where it works as a transcription factor utilising its basic helix loop helix (bHLH) domain. In the nucleus STRA8 binds promoters of multiple meiotic genes while also acting on its own promoter in a feedback loop [70]. However, STRA8 is not the only transcription factor required for meiosis initiation. SOHLH1, another bHLH containing transcription factor, interacts with STRA8 to activate some of the early meiotic genes crucial for both male and female meiosis initiation as well as synaptonemal complex formation and homologous recombination [71–73]. Furthermore, more recently, MEIOSIN was reported to be a direct downstream target of RA signalling [74].

RA is synthesised by three major retinaldehyde dehydrogenases (RALDH 1, 2, 3), in which RALDH2 and 3 are known to be the major sources of RA [65]. However, the mechanisms of STRA8 driven meiotic initiation remain rather ambiguous. Teletin et al. [65] reported that *STRA8* was expressed in the spermatocytes independently of RA signalling despite *RALDH2* and *RALDH3* being depleted. Similar results were observed in murine

fetal ovaries [75], indicating that RA may not be indispensable for *STRA8* expression, or perhaps that RALDH1 is the favourable source of RA for meiotic entry [76]. In the fetal ovary meiotic entry is regulated primarily by RA signalling. However, Bone Morphogenetic Protein (BMP) signalling is also crucial for meiosis-specific transcriptional regulation [77], which is established in the PGCs. Within this network, ZGLP1, a direct downstream effector of BMP4 signalling, is able to switch on repressed bivalent genes that contribute to entering oogenesis [78]. Moreover, *STRA8* can be activated by BMP2 expression as a downstream effector of Wnt/ β -catenin signalling, which regulates timely initiation of meiosis in female PGCs [79,80].

Conversely, mitotic division in male PGCs halts at E13.5 and meiosis is delayed until puberty. Meiotic inhibitors such as *NANOS2* and *DMRT1* maintain male germ cells in mitotic arrest stage and prevent them from entry into meiosis [81,82]. Recently evidence has emerged that *NANOS2* is a major regulator of initiating and maintaining mitotic arrest in a post-transcription regulation manner [83]. Furthermore, delays in male meiosis can be imposed by multiple RA repressors in order to prevent pre-mature meiotic onset. For instance, *FGF9* signalling plays a role in inhibiting *STRA8* expression in mice [84]. Moreover, a metabolising enzyme *CYP26B1* expressed in Sertoli cells within the fetal testis degrades RA [85]. Consequently, upon puberty, *DMRT1* was found to be diminished during zygotene and pachytene in human adult testis [82]. Similarly, *CYP26b1* is down-regulated in Sertoli cells prior to the onset of meiosis [86], followed by the expression of meiotic markers including *DMC1*, *STRA8* and *SYCP3* and the reduction in pluripotent markers within the adult testis, which mark meiotic entry.

Interestingly, although meiosis initiation is regulated by multiple signalling pathways within multicellular organisms, it has been reported that nutrient restriction and RA stimulation can activate the meiotic program through activation of a set of key transcription factors *in vitro* [87]. This observation is reminiscent of meiosis initiation in yeast, which is primarily nutrient and metabolism-driven. Expression of *IME1* upon nutrient starvation in yeast triggers the activation of meiotic genes such as *Spo11*, *Hop1* (*HORMAD1* in human) and *Dmc1* [88], which are also major meiotic regulators of double strand break formation, a crucial step for meiosis [82]. Similarly, transcriptional activation of the majority of meiotic regulators also requires additional cues such as RA signalling. Aforementioned signalling networks highlight the need for nutrient sensing mechanism conservation in meiosis initiation all the way from single cell organisms to mammals.

Epigenetic regulation of meiotic gene activation in mouse PGCs

To prepare for meiotic entry and acquire meiotic and developmental competence, global transcriptional changes are regulated differentially between the sexes. Transcriptional changes executed through global epigenetic reprogramming are most abrupt during the transition from PGCs to primary oocytes/spermatocytes [89,90]. This is evidenced by the global chromatin reorganisation and demethylation at E13.5 in mice which lead to large scale meiotic and embryonic gene activation [49]. At the chromatin level, removal of the Polycomb Repressive Complex 1 (PRC1) plays a crucial role in timely meiosis initiation [91]. The Polycomb Groups (PcGs) are chromatin remodellers responsible for transcriptional repression. PcGs contain different catalytic subunits allowing them to deposit repressive histone marks. The PRC1 central subunit, *Rnf2*, is a ubiquitin ligase responsible for inducing H2A ubiquitination. *Rnf2* deletion gives rise to global reduction in UbH2A and de-repression of a subset of meiotic prophase genes such as *Stra8*, *Sycp3*, *Rec8* and *Hormad2* [91]. Moreover, meiotic initiator *STRA8* is also a direct target of *Rnf2* while PRC1 plays a role in antagonising RA signalling and suppressing *STRA8* transcriptional activities in PGCs. *Rnf2* depletion sensitises PGCs to RA signalling, indicating that down-regulation of PRC1-mediated transcriptional repression is a prerequisite to meiotic entry [91]. In fact, the concurrence of DNA demethylation and PRC1 erasure may be prerequisite for potentiating germline gene expression. Hill et al. identified a subset of genes that are activated during PGC epigenetic reprogramming which are referred to as the Germline Reprogramming Responsive (GRR) genes. These GRR genes were activated after dual depletion of 5mC and PRC1 in both male and female PGCs at E13.5 [92].

It is important to note that histone replacement occurs prior to meiotic entry during PGC development [93]. To establish specific histone modifications for meiotic gene activation, large scale chromatin remodelling via histone displacement takes place after global DNA methylation. Histone chaperones NAP1 disassemble and remove core linker histone H1, followed by extraction of H2AZ [94]. Consequently, core repressive marks such as H2A/H4R3me2, H3K9me3, H3K27me3 and H3K9ac are diminished at E11.5, resulting in a large proportion of open chromatin [93]. Notably, this mechanism is likely to be sex-specific. Ueda et al. [95] demonstrated that

male-specific H3 variant H3t replaces H3.1 before entry into spermatogenesis and is more prone to inducing an open chromatin structure *in vitro*. These epigenetic mechanisms contribute to the priming of PGCs for meiotic gene activation.

Future prospects in meiosis research

The challenge presented by the reductive and final nature of meiosis, and the impossibility of a true meiotic cell lines, has hampered our ability to easily manipulate individual components of the meiotic process. As a result, molecular mechanisms of meiosis have mostly been studied using singled cell organisms. However, although a number of pathways are conserved between the aforementioned model species, it is essential to understand specific mechanisms that are required for timely meiosis initiation among species, particularly in higher organisms. Therefore, mounting efforts have been invested into *in vitro* induction of meiotic entry utilising spermatogonial stem cells, induced pluripotent stem cells and embryonic stem cells, which can be differentiated into PGC-Like Cells (PGCLC) [96]. Hikabe et al. developed an *in vitro* culture system to induce meiotic entry and map the entire cycles of germ line maturation by co-culturing the murine PGCLCs with gonadal somatic cells. The resulting male and female mature gametes were fertile and gave rise to viable pups [97]. In addition, Hamazaki et al. [98] have successfully reconstituted the transcription networks required for oocyte maturation using mPGCLCs. In these PGCLCs, the essential transcription factors and epigenetic configurations required for meiotic initiation largely recapitulated those of mouse PGCs [99].

Although these models cannot fully replicate the *in vivo* conditions, they can be used to investigate environmental cues for meiotic entry. For example, Shimamoto et al. [100] reported that primary oocytes bypassed meiotic arrest at diplotene stage in prophase I without inducing hypoxia, indicating that hypoxia serves as one of the major factors maintaining oocyte dormancy which is mediated by nuclear localisation of FOXO3. Utilisation of these tools has led to recent appreciation of the evolutionarily conserved role of nutrient-deprivation in meiosis initiation which, combined with careful studies of meiosis-activating transcriptional networks, has led to landmark discoveries allowing experimental modelling of meiotic initiation in murine cells [78,87,97,98]. Now that meiosis initiation can be modelled and manipulated, the direct contribution of specific cellular process during meiosis initiation must be established.

The role of nutrient sensing in meiotic initiation

In fission yeast, autophagy is likely to be an important player during meiotic initiation as it is known to be activated upon nutrient deprivation [101]. Autophagy is crucial for reserving energy in response to cellular stress conditions such as nutrient and oxygen starvation. In both fission and budding yeast meiotic entry fails if autophagy is deficient [102,103]. Autophagy acts to degrade major meiotic entry inhibitors allowing the cell to enter meiosis [104]. Furthermore, in *Drosophila*, even though nutrient starvation leads to a reduction in oocyte production, autophagy activation in follicle cells is essential for oogenesis due to its role in supporting signalling between nurse cells and oocytes [105]. Moreover, mouse models lacking an essential autophagy gene, AuTophagy related 7 (Atg7), are infertile even though specific mechanism remains unclear [106,107]. However, in mammals STRA8 represses autophagy by binding the promoter of Nr1d1, which in turn leads to repressing its downstream target ULK1, an autophagy initiator, highlighting the requirement for the suppression of autophagy during meiosis initiation [108]. It is likely that metabolic stress, in concert with RA signalling, act to activate meiosis in mammals [108]. As mentioned in previous sections, Wang et al. [87] reported based on the scRNA-seq data that nutrient starvation is likely to be the metabolic stress inducing switching from glycolysis to mitochondrial oxidative phosphorylation. *In vivo*, this metabolic stress, which in the male is further contributed to by the blood-testis barrier (BTB), is required for SSC differentiation [109,110]. In female ovary, this stress could be created by germ cell cysts restricting nutrient access. Although the role for nutrient deprivation in meiosis is potentially conserved it is yet to be fully understood in mammals. It can be hypothesised that the suppression of autophagy might be important in maintaining meiotic DSBs during prophase I as autophagy plays a role in DNA damage repair [111]. The master regulator of autophagy, mTORC1, is found to be crucial during meiotic onset. The mTOR pathway is involved in many cellular processes such as cell growth, proliferation, protein synthesis, nutrient sensing and autophagy [112] with its role in meiotic entry largely conserved from yeast to mammals [113]. In both yeast and female drosophila, reduction in TORC1 expression in response to nutrient starvation is needed for mitotic-meiotic switch [114,115]. In *C. elegans*, stem cells proliferation and maintenance if the stem cell pool depend on autophagy via nutrient sensing pathways including TGF β /DAF-7 signalling, ribosomal protein S6 kinase (S6K) and insulin IGF-1-like signalling (IIS) that are

involved in TOR signalling [116–118]. However, the role of autophagy in meiotic entry in *C. elegans* is not well-explored. In mammals, however, TORC1 signalling is crucial for SSCs differentiation [119]. Suppression of mTORC1 activators is required for male mitotic arrest in PGCs [83], which could prime male germ cells for meiotic entry. Sahin et al. [120] demonstrated that mTOR is a target of RA signalling required for STRA8 expression during male meiotic onset. These observations suggest that in mammals, meiotic initiation requires mTOR1 expression and hence suppression of autophagy as these two factors are mutually exclusive. Consequently, metabolic stresses that acts on non-autophagic pre-meiotic cells, in parallel with RA stimulation, may be prerequisite for meiotic entry.

Emerging links between meiotic initiation and oncogenesis

Recently uncovered links between nutrient deprivation and mammalian meiosis [87] raise a series of important questions. For many years, dogma stated that the Weismann barrier (a distinction between ‘immortal’ germ cell lineages that generate gametes and ‘disposable’ somatic cells) explained the very tight control of meiotic gene expression, which was thought to limit transcription exclusively to germ cells. However, following technical advances in transcriptomic and proteomic analysis it is becoming increasingly clear that meiotic gene expression does occur in the soma, and in cancer cells. The first meiotic genes reported expressed in cancers were found to be immunogenic and were consequently classified as Cancer Testis Antigens (CTA), however with increasing number of CTAs identified it has become clear that these are not limited to testis specific proteins, and that not all of them are immunogenic. To address this issue, the term Germ Cell Cancer Genes (GCCG) has been proposed to describe the variety of meiotic genes re-expressed in cancer cells [121]. Currently over a thousand GCCGs have been reported widely re-expressed across cancer types, highlighting the importance of this process (see below) [105,121–124].

The presence of meiotic proteins in cells other than germ also brings into to question the validity of the Weismann barrier hypothesis [121,122,125], which now needs to be evaluated experimentally through a combination of cell signalling, epigenetic and transcriptional analyses. Indeed, it is now timely to consider whether genes required for meiosis specific processes might also possess non-meiotic functions; such ‘moonlighting’

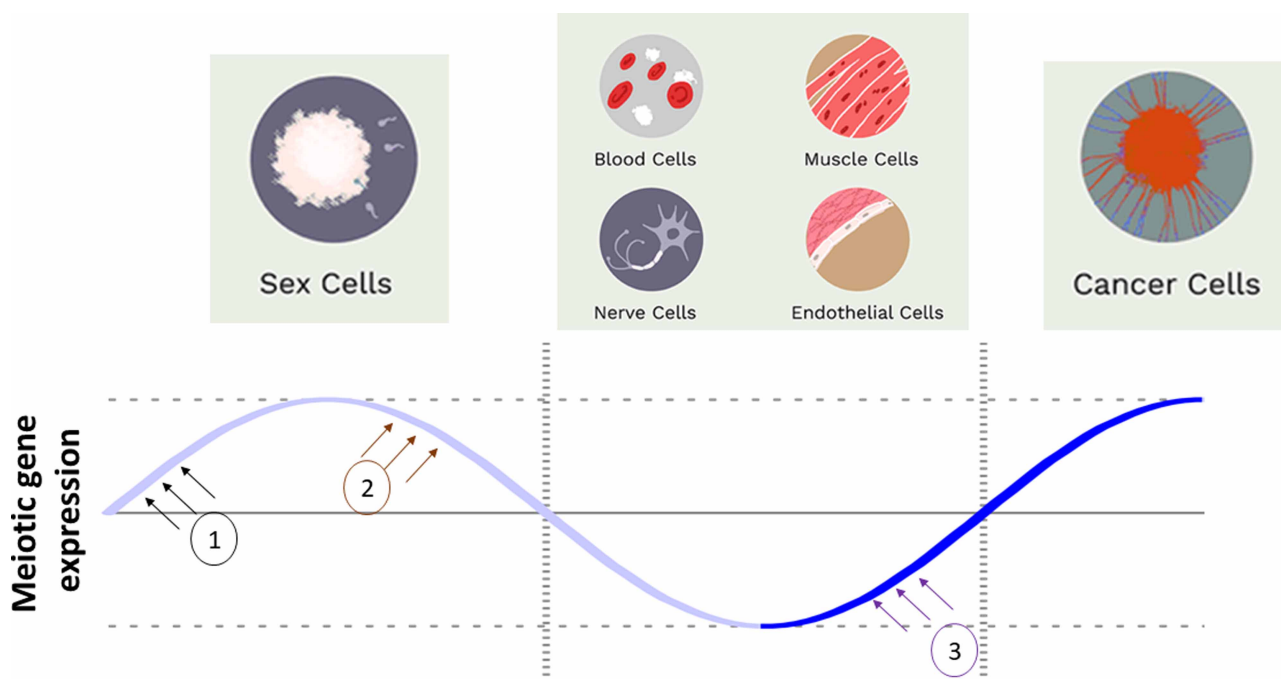


Figure 2. Expression of genes understood to be exclusively meiotic is assumed tightly regulated and silenced in healthy somatic cells.

However, we still lack detailed mechanistic understanding of the machinery that turns on meiotic gene expression during meiosis initiation (1), turns expression off during the meiotic-to-mitotic transition (2), and (3) the factors responsible for aberrant reactivation of meiotic gene expression in, for example, cancer cells.

functions have been characterised for a number of human mitotic proteins including the HSP family members, clathrin and dynein [126]. Recently, we identified a novel, ‘moonlighting’ function for the meiotic protein TEX12 (Testis Expressed 12) within microtubule organising centres, organelles that are fundamental for meiosis and mitosis [122]. TEX12 gene transcription and protein expression were originally believed to be exclusively meiotic with expressed protein restricted to the meiosis-specific synaptonemal complex, which assembles between aligned homologous chromosomes during prophase to facilitate DNA recombination. Similar moonlighting functions have been previously reported for other meiotic proteins such as Sme4 and Hei10 [127,128]. Furthermore, we found TEX12 to be a GCGG that is widely expressed in ~15% of cancer patients [122]. Frequent expression of TEX12, and other meiotic genes in human cancers highlights that the process of oncogenesis might recreate, or be triggered by, some of the conditions characteristic of meiotic initiation resulting in large scale activation of the meiotic transcriptome. It appears possible, for example, that this might be linked to nutrient deprivation within solid tumours and associated changes in autophagy signalling. It is also plausible that some meiotic genes are endogenously expressed in distinct cell and tissue types, for example in the brain, where the blood-brain barrier might recapitulate physiological processes that are known to activate meiosis in the testis, such as nutrient starvation experienced as a consequence of the blood-testis barrier (Figure 2). This is reminiscent of angiogenesis and shaping of the tumour microenvironment where nutrients are generally deprived [129]. In the tumour microenvironment, cancer cells require high nutrient uptake to maintain survival and proliferation during tumour initiation. mTOR complexes are known to play a role as amino acid sensors, allowing cancer cells to adapt to the harsh environment by epigenetic shift and metabolic reprogramming [130,131]. This recapitulates the amino acid restriction within the gonad that surrounds germ cells which could explain thereactivation of meiotic genes [87,132].

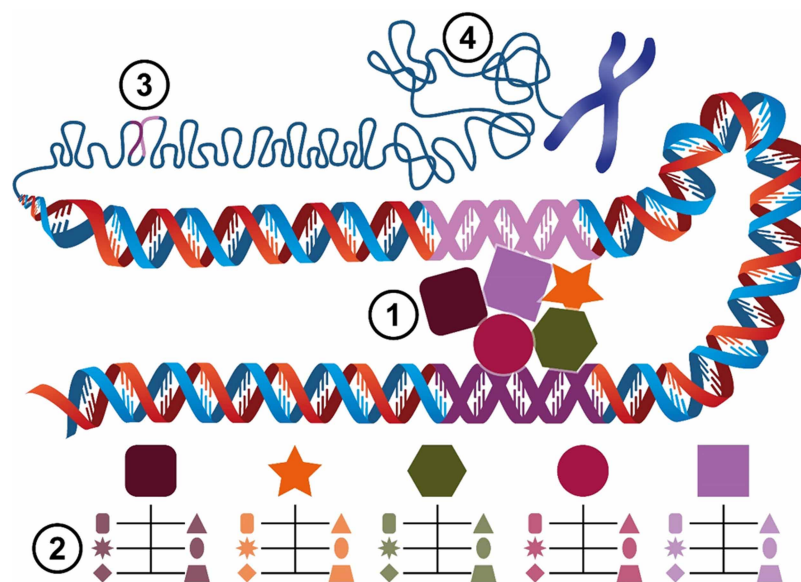


Figure 3. Unanswered questions about meiotic gene activation.

(1) At the DNA level, interactions between the gene promoter (pink) and enhancer (purple) regions controls recruitment of transcription regulating machinery including transcription factors/repressors and their cofactors. What are the cell type specific meiotic promoter binding proteins and how do they regulate exclusively meiotic expression? (2) The availability of each meiotic promoter binding protein (shown as (1)) is affected by a network of specific cofactors (rectangle), kinases (star), phosphatases (diamond), E3s (triangle), deubiquitinating enzymes (oval) and others (trapezoid). What is the complete network of these meiotic expression master regulators? (3) Gene expression is affected by distal enhancers (purple) and the 3D genome architecture which controls their ability to interact with gene promoters (pink) to facilitate the recruitment of transcriptional machinery. What are the cell type specific distal enhancers of meiotic genes? (4) At a higher level of genome compaction large topologically associated domains (TADs) become the units of chromatin and a position of the gene within the TADs can affect its expression. What are the cell type specific TAD domain positions of meiotic genes and what role does the TAD positioning play in regulating the expression of meiotic genes?

Notably, chromatin reorganisation can be affected by nutrient availability. Kirmes et al. [133] demonstrated that chromatin compaction and alteration of nuclear architecture can be induced by nutrient depletion. These changes could lead to down-regulation of meiotic gene suppressors during oncogenesis similarly to what happens at meiotic entry. In fission yeast, TORC1 signalling induces a facultative heterochromatin state at meiotic genes in a nutrient sensitive manner. Inhibition of TOR leads to disassembly of a heterochromatin islands forming complex [134]. Similar mechanisms may be exploited during meiotic gene re-activation in cancer, suggesting that global epigenetic changes play a role in meiotic gene activation during oncogenesis.

Collectively, metabolic stresses and mTOR signalling appear to be crucial for both meiotic gene expression and oncogenesis. Further reviews exploring the role of TOR pathways across different species and in cancer can be found here [113,130]. Emerging links between meiotic initiation and oncogenesis require further investigation and conserved meiotic entry pathways discussed in this review might provide novel insights to cancer initiation.

Conclusion

Now that experimental models for murine meiotic initiation have been established, it is timely to revisit the Weismann barrier hypothesis in order to understand how concerted silencing of meiotic gene transcription in the autosomes is regulated and synchronised and also to define the contribution of germ transcriptome to the soma (Figure 3) under both normal and pathological conditions. This leads us to pose several key questions pertaining to DNA superstructure and the specific machinery used to ensure meiosis-specific transcription in response to a variety of cell signalling mechanisms (Figure 3). Understanding these processes will help drive a more complete understanding of the links between meiosis initiation, transcription and cancer.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Open Access

Open access for this article was enabled by the participation of University of Liverpool in an all-inclusive *Read & Publish* pilot with Portland Press and the Biochemical Society under a transformative agreement with JISC.

Acknowledgements

U.L.M. was supported by the University of Liverpool Tenure Track programme, the Royal Society Research Grant RG170342 and by the Wellcome Trust 204822/Z/16/Z. I.F.S. was supported by a joint University of Liverpool - A* PhD programme. R.P. was supported by the BBSRC NLD DTP PhD programme. W.W.T. is supported by the National Research Foundation (NRF) Singapore, under the NRF fellowship (NRF-NRFF2016-06) and the National Medical Research Council (NMRC; OFIRG19nov0015). The authors thank Professor Patrick A. Eyers for insights, advice and support during the writing of this review.

Abbreviations

BMP, bone morphogenic protein; CB, cytoblast; DTC, distal tip cell; FSH, follicle stimulating hormone; GB, gonialblast cell; GRR, germline reprogramming responsive; GSC, germline stem cells; IME1, Inducer of MEiosis 1; LH, luteinizing hormone; MPF, maturation promoting factor; PGCs, primordial germ cells; PKA, Protein Kinase A; PRC1, Polycomb Repressive Complex 1; RA, retinoic acid; RXR, retinoid X receptors; SSCs, spermatogonial stem cells; TORC1, Target Of Rapamycin Complex 1.

References

- 1 Mitchell, A.P. (1994) Control of meiotic gene expression in *Saccharomyces cerevisiae*. *Microbiol. Rev.* **58**, 56–70 <https://doi.org/10.1128/mr.58.1.56-70.1994>
- 2 Covitz, P.A. and Mitchell, A.P. (1993) Repression by the yeast meiotic inhibitor RME1. *Genes Dev.* **7**, 1598–1608 <https://doi.org/10.1101/gad.7.8.1598>
- 3 Chu, S., DeRisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P.O. et al. (1998) The transcriptional program of sporulation in budding yeast. *Science* **282**, 699–705 <https://doi.org/10.1126/science.282.5389.699>
- 4 Honigberg, S.M. and Purnapatre, K. (2003) Signal pathway integration in the switch from the mitotic cell cycle to meiosis in yeast. *J. Cell Sci.* **116**, 2137–2147 <https://doi.org/10.1242/jcs.00460>
- 5 Nachman, I., Regev, A. and Ramanathan, S. (2007) Dissecting timing variability in yeast meiosis. *Cell* **131**, 544–556 <https://doi.org/10.1016/j.cell.2007.09.044>
- 6 van Werven, F.J., Neuert, G., Hendrick, N., Lardenois, A., Buratowski, S., van Oudenaarden, A. et al. (2012) Transcription of two long noncoding RNAs mediates mating-type control of gametogenesis in budding yeast. *Cell* **150**, 1170–1181 <https://doi.org/10.1016/j.cell.2012.06.049>

- 7 Mitchell, A.P. and Herskowitz, I. (1986) Activation of meiosis and sporulation by repression of the RME1 product in yeast. *Nature* **319**, 738–742
<https://doi.org/10.1038/319738a0>
- 8 Sagee, S., Sherman, A., Shenhar, G., Robzyk, K., Ben-Doy, N., Simchen, G. et al. (1998) Multiple and distinct activation and repression sequences mediate the regulated transcription of IME1, a transcriptional activator of meiosis-specific genes in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **18**, 1985–1995
<https://doi.org/10.1128/MCB.18.4.1985>
- 9 Weidberg, H., Moretto, F., Spedale, G., Amon, A. and van Werven, F.J. (2016) Nutrient control of yeast gametogenesis is mediated by TORC1, PKA and energy availability. *PLoS Genet.* **12**, e1006075
<https://doi.org/10.1371/journal.pgen.1006075>
- 10 Malathi, K., Xiao, Y. and Mitchell, A.P. (1997) Interaction of yeast repressor-activator protein Ume6p with glycogen synthase kinase 3 homolog Rim1p. *Mol. Cell. Biol.* **17**, 7230–7236
<https://doi.org/10.1128/MCB.17.12.7230>
- 11 Guttman-Raviv, N., Martin, S. and Kassir, Y. (2002) Ime2, a meiosis-specific kinase in yeast, is required for destabilization of its transcriptional activator, Ime1. *Mol. Cell. Biol.* **22**, 2047–2056
<https://doi.org/10.1128/MCB.22.7.2047-2056.2002>
- 12 Wannige, C.T., Kulasiri, D. and Samarasinghe, S. (2014) A nutrient dependant switch explains mutually exclusive existence of meiosis and mitosis initiation in budding yeast. *J. Theor. Biol.* **341**, 88–101
<https://doi.org/10.1016/j.jtbi.2013.09.030>
- 13 Kunitomo, H., Higuchi, T., Iino, Y. and Yamamoto, M. (2000) A zinc-finger protein, Rst2p, regulates transcription of the fission yeast *ste11(+)* gene, which encodes a pivotal transcription factor for sexual development. *Mol. Biol. Cell* **11**, 3205–3217
<https://doi.org/10.1091/mbc.11.9.3205>
- 14 Toru, H., Yoshinori, W. and Masayuki, Y. (2002) Protein kinase A regulates sexual development and gluconeogenesis through phosphorylation of the Zn finger transcriptional activator Rst2p in fission yeast. *Mol. Cell. Biol.* **22**, 1–11
<https://doi.org/10.1128/MCB.22.1.1-11.2002>
- 15 Kitamura, K., Katayama, S., Dhut, S., Sato, M., Watanabe, Y., Yamamoto, M. et al. (2001) Phosphorylation of Mei2 and Ste11 by Pat1 kinase inhibits sexual differentiation via ubiquitin proteolysis and 14-3-3 protein in fission yeast. *Dev. Cell* **1**, 389–399
[https://doi.org/10.1016/S1534-5807\(01\)00037-5](https://doi.org/10.1016/S1534-5807(01)00037-5)
- 16 Li, L. and Xie, T. (2005) Stem cell niche: structure and function. *Annu. Rev. Cell Dev. Biol.* **21**, 605–631
<https://doi.org/10.1146/annurev.cellbio.21.012704.131525>
- 17 Lin, H. and Spradling, A.C. (1993) Germline stem cell division and Egg chamber development in transplanted *Drosophila* germaria. *Dev. Biol.* **159**, 140–152
<https://doi.org/10.1006/dbio.1993.1228>
- 18 de Cuevas, M. and Matunis, E.L. (2011) The stem cell niche: lessons from the *Drosophila* testis. *Development* **138**, 2861–2869
<https://doi.org/10.1242/dev.056242>
- 19 Coutelis, J.B., Petzoldt, A.G., Spéder, P., Suzanne, M. and Noselli, S. (2008) Left–right asymmetry in *Drosophila*. *Semin. Cell Dev. Biol.* **19**, 252–262
<https://doi.org/10.1016/j.semcdb.2008.01.006>
- 20 Demarco, R.S., Eikenes, Å.H., Haglund, K. and Jones, D.L. (2014) Investigating spermatogenesis in *Drosophila melanogaster*. *Methods* **68**, 218–227
<https://doi.org/10.1016/j.ymeth.2014.04.020>
- 21 Insko, M.L., Leon, A., Tam, C.H., McKearin, D.M. and Fuller, M.T. (2009) Accumulation of a differentiation regulator specifies transit amplifying division number in an adult stem cell lineage. *Proc. Natl Acad. Sci. U.S.A.* **106**, 22311–6
<https://doi.org/10.1073/pnas.0912454106>
- 22 Spradling, A., Fuller, M.T., Braun, R.E. and Yoshida, S. (2011) Germline stem cells. *Cold Spring Harb. Perspect. Biol.* **3**, a002642
<https://doi.org/10.1101/cshperspect.a002642>
- 23 Gilboa, L. and Lehmann, R. (2006) Soma–germline interactions coordinate homeostasis and growth in the *Drosophila* gonad. *Nature* **443**, 97–100
<https://doi.org/10.1038/nature05068>
- 24 Hughes, S.E., Miller, D.E., Miller, A.L. and Hawley, R.S. (2018) Female meiosis: synapsis, recombination, and segregation in *Drosophila melanogaster*. *Genetics* **208**, 875–908
<https://doi.org/10.1534/genetics.117.300081>
- 25 Zhong, W. and Haifan, L. (2004) Nanos maintains germline stem cell self-renewal by preventing differentiation. *Science* **303**, 2016–2019
<https://doi.org/10.1126/science.1093983>
- 26 Hirsh, D., Oppenheim, D. and Klass, M. (1976) Development of the reproductive system of *Caenorhabditis elegans*. *Dev. Biol.* **49**, 200–219
[https://doi.org/10.1016/0012-1606\(76\)90267-0](https://doi.org/10.1016/0012-1606(76)90267-0)
- 27 Hodgkin, J., Horvitz, H.R. and Brenner, S. (1979) Nondisjunction mutants of the nematode *Caenorhabditis elegans*. *Genetics* **91**, 67–94
<https://doi.org/10.1093/genetics/91.1.67>
- 28 Crittenden, S.L., Leonhard, K.A., Byrd, D.T. and Kimble, J. (2006) Cellular analyses of the mitotic region in the *Caenorhabditis elegans* adult germ line. *Mol. Biol. Cell* **17**, 3051–3061
<https://doi.org/10.1091/mbc.e06-03-0170>
- 29 Mohammad, A., Vanden Broek, K., Wang, C., Daryabeigi, A., Jantsch, V., Hansen, D. et al. (2018) Initiation of meiotic development is controlled by three post-transcriptional pathways in *Caenorhabditis elegans*. *Genetics* **209**, 1197–1224
<https://doi.org/10.1534/genetics.118.300985>
- 30 Kimble, J.E. and White, J.G. (1981) On the control of germ cell development in *Caenorhabditis elegans*. *Dev. Biol.* **81**, 208–219
[https://doi.org/10.1016/0012-1606\(81\)90284-0](https://doi.org/10.1016/0012-1606(81)90284-0)
- 31 Killian, D.J. and Hubbard, E.J.A. (2005) *Caenorhabditis elegans* germline patterning requires coordinated development of the somatic gonadal sheath and the germ line. *Dev. Biol.* **279**, 322–335
<https://doi.org/10.1016/j.ydbio.2004.12.021>
- 32 Fox, P.M. and Schedl, T. (2015) Analysis of germline stem cell differentiation following loss of GLP-1 notch activity in *Caenorhabditis elegans*. *Genetics* **201**, 167–184
<https://doi.org/10.1534/genetics.115.178061>
- 33 Hansen, D., Wilson-Berry, L., Dang, T. and Schedl, T. (2004) Control of the proliferation versus meiotic development decision in the *C. elegans* germline through regulation of GLD-1 protein accumulation. *Development* **131**, 93–104
<https://doi.org/10.1242/dev.00916>
- 34 Maller, J.L. (2012) Pioneering the *Xenopus* oocyte and egg extract system. *J. Biol. Chem.* **287**, 21640–21653
<https://doi.org/10.1074/jbc.X112.371161>
- 35 Gautier, J., Minshull, J., Lohka, M., Glotzer, M., Hunt, T. and Maller, J.L. (1990) Cyclin is a component of maturation-promoting factor from *xenopus*. *Cell* **60**, 487–494
[https://doi.org/10.1016/0092-8674\(90\)90599-A](https://doi.org/10.1016/0092-8674(90)90599-A)
- 36 Eyers, P.A., Liu, J., Hayashi, N.R., Lewellyn, A.L., Gautier, J. and Maller, J.L. (2005) Regulation of the G₂/M transition in *Xenopus* oocytes by the cAMP-dependent protein kinase. *J. Biol. Chem.* **280**, 24339–24346
<https://doi.org/10.1074/jbc.M412442200>
- 37 Dupré, A., Daldello, E.M., Nairn, A.C., Jessus, C. and Haccard, O. (2014) Phosphorylation of ARPP19 by protein kinase A prevents meiosis resumption in *Xenopus* oocytes. *Nat. Commun.* **5**, 3318
<https://doi.org/10.1038/ncomms4318>
- 38 Aicha, G.-A., Jean-Claude, L., Andrew, B., Suzanne, V., Jean-Marc, S., Estelle, B. et al. (2010) The substrate of greatwall kinase, Arpp19, controls mitosis by inhibiting protein phosphatase 2A. *Science* **330**, 1673–1677
<https://doi.org/10.1126/science.1197048>

- 39 Gautier, J., Norbury, C., Lohka, M., Nurse, P. and Maller, J. (1988) Purified maturation-promoting factor contains the product of a *Xenopus* homolog of the fission yeast cell cycle control gene *cdc2⁺*. *Cell* **54**, 433–439 [https://doi.org/10.1016/0092-8674\(88\)90206-1](https://doi.org/10.1016/0092-8674(88)90206-1)
- 40 Yew, N., Mellini, M.L., Martinez, C.K. and Vande, W.G. (1992) Meiotic initiation by the *mos* protein in *Xenopus*. *Nature* **355**, 649–652 <https://doi.org/10.1038/355649a0>
- 41 Gurdon, J. and Hopwood, N. (2000) The introduction of *Xenopus laevis* into developmental biology: of empire, pregnancy testing and ribosomal genes. *Int. J. Dev. Biol.* **44**, 43–50 PMID:10761846
- 42 Meneau, F., Dupré, A., Jessus, C. and Daldello, E.M. (2020) Translational control of *Xenopus* oocyte meiosis: toward the genomic Era. *Cells* **9**, 1502 <https://doi.org/10.3390/cells9061502>
- 43 Philpott, A. and Yew, P.R. (2008) The *Xenopus* cell cycle: an overview. *Mol. Biotechnol.* **39**, 9–19 <https://doi.org/10.1007/s12033-008-9033-z>
- 44 Zafar, M.I., Lu, S. and Li, H. (2021) Sperm-oocyte interplay: an overview of spermatozoon's role in oocyte activation and current perspectives in diagnosis and fertility treatment. *Cell Biosci.* **11**, 4 <https://doi.org/10.1186/s13578-020-00520-1>
- 45 Barroso, G., Valdespin, C., Vega, E., Kershenovich, R., Avila, R., Avendaño, C. et al. (2009) Developmental sperm contributions: fertilization and beyond. *Fertil. Steril.* **92**, 835–848 <https://doi.org/10.1016/j.fertnstert.2009.06.030>
- 46 Simerly, C., Manil-Ségalen, M., Castro, C., Hartnett, C., Kong, D., Verlhac, M.-H. et al. (2018) Separation and loss of centrioles from primordial germ cells to mature oocytes in the mouse. *Sci. Rep.* **8**, 12791 <https://doi.org/10.1038/s41598-018-31222-x>
- 47 Avidor-Reiss, T., Khire, A., Fishman, E.L. and Jo, K.H. (2015) Atypical centrioles during sexual reproduction. *Front. cell Dev. Biol.* **3**, 21 <https://doi.org/10.3389/fcell.2015.00021>
- 48 McLaren, A. (2003) Primordial germ cells in the mouse. *Dev. Biol.* **262**, 1–15 [https://doi.org/10.1016/S0012-1606\(03\)00214-8](https://doi.org/10.1016/S0012-1606(03)00214-8)
- 49 Hajkova, P., Erhardt, S., Lane, N., Haaf, T., El-Maarri, O., Reik, W. et al. (2002) Epigenetic reprogramming in mouse primordial germ cells. *Mech. Dev.* **117**, 15–23 [https://doi.org/10.1016/S0925-4773\(02\)00181-8](https://doi.org/10.1016/S0925-4773(02)00181-8)
- 50 Oduwole, O.O., Peltoketo, H. and Huhtaniemi, I.T. (2018) Role of follicle-stimulating hormone in spermatogenesis. *Front. Endocrinol.* **9**, 763 <https://doi.org/10.3389/fendo.2018.00763>
- 51 Zirkin, B.R. and Papadopoulos, V. (2018) Leydig cells: formation, function, and regulation. *Biol. Reprod.* **99**, 101–111 <https://doi.org/10.1093/biore/roy059>
- 52 Holstein, A.-F., Schulze, W. and Davidoff, M. (2003) Understanding spermatogenesis is a prerequisite for treatment. *Reprod. Biol. Endocrinol.* **1**, 107 <https://doi.org/10.1186/1477-7827-1-107>
- 53 Griswold, M.D. (2016) Spermatogenesis: the commitment to meiosis. *Physiol. Rev.* **96**, 1–17 <https://doi.org/10.1152/physrev.00013.2015>
- 54 Jan, S.Z., Vormer, T.L., Jongejan, A., Röling, M.D., Silber, S.J., de Rooij, D.G. et al. (2017) Unraveling transcriptome dynamics in human spermatogenesis. *Development* **144**, 3659–3673 <https://doi.org/10.1242/dev.152413>
- 55 Kimmins, S., Kotaja, N., Davidson, I. and Sassone-Corsi, P. (2004) Testis-specific transcription mechanisms promoting male germ-cell differentiation. *Reproduction* **128**, 5–12 <https://doi.org/10.1530/rep.1.00170>
- 56 Wouters-Tyrou, D., Martinage, A., Chevallier, P. and Sautière, P. (1998) Nuclear basic proteins in spermiogenesis. *Biochimie* **80**, 117–128 [https://doi.org/10.1016/S0300-9084\(98\)80018-7](https://doi.org/10.1016/S0300-9084(98)80018-7)
- 57 Jaffe, L.A. and Egbert, J.R. (2017) Regulation of mammalian oocyte meiosis by intercellular communication within the ovarian follicle. *Annu. Rev. Physiol.* **79**, 237–260 <https://doi.org/10.1146/annurev-physiol-022516-034102>
- 58 Clift, D. and Schuh, M. (2013) Restarting life: fertilization and the transition from meiosis to mitosis. *Nat. Rev. Mol. Cell Biol.* **14**, 549–562 <https://doi.org/10.1038/nrm3643>
- 59 Jones, K.T. (2004) Turning it on and off: M-phase promoting factor during meiotic maturation and fertilization. *Mol. Hum. Reprod.* **10**, 1–5 <https://doi.org/10.1093/molehr/gah009>
- 60 Madgwick, S. and Jones, K.T. (2007) How eggs arrest at metaphase II: MPF stabilisation plus APC/C inhibition equals cytostatic factor. *Cell Div.* **2**, 4 <https://doi.org/10.1186/1747-1028-2-4>
- 61 De La Fuente, R., Viveiros, M.M., Burns, K.H., Adashi, E.Y., Matzuk, M.M. and Eppig, J.J. (2004) Major chromatin remodeling in the germinal vesicle (GV) of mammalian oocytes is dispensable for global transcriptional silencing but required for centromeric heterochromatin function. *Dev. Biol.* **275**, 447–458 <https://doi.org/10.1016/j.ydbio.2004.08.028>
- 62 Medvedev, S., Pan, H. and Schultz, R.M. (2011) Absence of MSY2 in mouse oocytes perturbs oocyte growth and maturation, RNA stability, and the transcriptome. *Biol. Reprod.* **85**, 575–583 <https://doi.org/10.1095/biolreprod.111.091710>
- 63 Stutz, A., Conne, B., Huarte, J., Gubler, P., Völkel, V., Flandin, P. et al. (1998) Masking, unmasking, and regulated polyadenylation cooperate in the translational control of a dormant mRNA in mouse oocytes. *Genes Dev.* **12**, 2535–2548 <https://doi.org/10.1101/gad.12.16.2535>
- 64 Spiller, C.M. and Bowles, J. (2015) Sex determination in mammalian germ cells. *Asian J. Androl.* **17**, 427–432 <https://doi.org/10.4103/1008-682X.150037>
- 65 Teletin, M., Vernet, N., Yu, J., Klopfenstein, M., Jones, J.W., Féret, B. et al. (2019) Two functionally redundant sources of retinoic acid secure spermatogonia differentiation in the seminiferous epithelium. *Development* **146**, dev170225 <https://doi.org/10.1242/dev.170225>
- 66 van Pelt, A.M. and de Rooij, D.G. (1991) Retinoic acid is able to reinitiate spermatogenesis in vitamin A-deficient rats and high replicate doses support the full development of spermatogenic cells. *Endocrinology* **128**, 697–704 <https://doi.org/10.1210/endo-128-2-697>
- 67 Koubova, J., Menke, D.B., Zhou, Q., Capel, B., Griswold, M.D. and Page, D.C. (2006) Retinoic acid regulates sex-specific timing of meiotic initiation in mice. *Proc. Natl Acad. Sci. U.S.A.* **103**, 2474–2479 <https://doi.org/10.1073/pnas.0510813103>
- 68 Anderson, E.L., Baltus, A.E., Roepers-Gajadien, H.L., Hassold, T.J., de Rooij, D.G., van Pelt, A.M.M. et al. (2008) *Stra8* and its inducer, retinoic acid, regulate meiotic initiation in both spermatogenesis and oogenesis in mice. *Proc. Natl Acad. Sci. U.S.A.* **105**, 14976–14980 <https://doi.org/10.1073/pnas.0807297105>
- 69 Soh, Y.Q.S., Junker, J.P., Gill, M.E., Mueller, J.L., van Oudenaarden, A. and Page, D.C. (2015) A gene regulatory program for meiotic prophase in the fetal ovary. *PLoS Genet.* **11**, e1005531 <https://doi.org/10.1371/journal.pgen.1005531>
- 70 Kojima, M.L., de Rooij, D.G. and Page, D.C. (2019) Amplification of a broad transcriptional program by a common factor triggers the meiotic cell cycle in mice. *eLife* **8**, e43738 <https://doi.org/10.7554/eLife.43738>
- 71 Velte, E.K., Niedenberger, B.A., Serra, N.D., Singh, A., Roa-DeLaCruz, L., Hermann, B.P. et al. (2019) Differential RA responsiveness directs formation of functionally distinct spermatogonial populations at the initiation of spermatogenesis in the mouse. *Development* **146**, dev173088 <https://doi.org/10.1242/dev.173088>

- 72 Desimio, M.G., Cesari, E., Sorrenti, M., De Felici, M. and Farini, D. (2021) Stimulated by retinoic acid gene 8 (STRA8) interacts with the germ cell specific bHLH factor SOHLH1 and represses c-KIT expression in vitro. *J Cell Mol Med.* **25**, 383–396 <https://doi.org/10.1111/jcmm.16087>
- 73 Li, Y., Qi, W., Liu, G., Du, B., Sun, Q., Zhang, X. et al. (2019) Sohlh1 is required for synaptonemal complex formation by transcriptionally regulating meiotic genes during spermatogenesis in mice. *Mol. Reprod. Dev.* **86**, 252–264 <https://doi.org/10.1002/mrd.23100>
- 74 Ishiguro, K., Matsuura, K., Tani, N., Takeda, N., Usuki, S., Yamane, M. et al. (2020) MEIOSIN directs the switch from mitosis to meiosis in mammalian germ cells. *Dev. Cell* **52**, 429–445.e10 <https://doi.org/10.1016/j.devcel.2020.01.010>
- 75 Kumar, S., Chatzi, C., Brade, T., Cunningham, T.J., Zhao, X. and Duester, G. (2011) Sex-specific timing of meiotic initiation is regulated by Cyp26b1 independent of retinoic acid signalling. *Nat. Commun.* **2**, 151 <https://doi.org/10.1038/ncomms1136>
- 76 Bowles, J., Feng, C.-W., Knight, D., Smith, C.A., Roeszler, K.N., Bagheri-Fam, S. et al. (2009) Male-specific expression of Aldh1a1 in mouse and chicken fetal testes: Implications for retinoid balance in gonad development. *Dev. Dyn.* **238**, 2073–2080 <https://doi.org/10.1002/dvdy.22024>
- 77 Miyauchi, H., Ohta, H., Nagaoka, S., Nakaki, F., Sasaki, K., Hayashi, K. et al. (2017) Bone morphogenetic protein and retinoic acid synergistically specify female germ-cell fate in mice. *EMBO J.* **36**, 3100–3119 <https://doi.org/10.15252/emj.201796875>
- 78 Nagaoka, S.I., Nakaki, F., Miyauchi, H., Nosaka, Y., Ohta, H., Yabuta, Y. et al. (2020) ZGLP1 is a determinant for the oogenic fate in mice. *Science* **367**, eaaw4115 <https://doi.org/10.1126/science.aaw4115>
- 79 Le Rolle, M., Massa, F., Siggers, P., Turchi, L., Loubat, A., Koo, B.-K. et al. (2021) Arrest of WNT/β-catenin signaling enables the transition from pluripotent to differentiated germ cells in mouse ovaries. *Proc. Natl Acad. Sci. U.S.A.* **118**, e2023376118 <https://doi.org/10.1073/pnas.2023376118>
- 80 Naillat, F., Prunskaitė-Hyryläinen, R., Pietilä, I., Sormunen, R., Jokela, T., Shan, J. et al. (2010) Wnt4/5a signalling coordinates cell adhesion and entry into meiosis during presumptive ovarian follicle development. *Hum. Mol. Genet.* **19**, 1539–1550 <https://doi.org/10.1093/hmg/ddq027>
- 81 Suzuki, A. and Saga, Y. (2008) Nanos2 suppresses meiosis and promotes male germ cell differentiation. *Genes Dev.* **22**, 430–435 <https://doi.org/10.1101/gad.1612708>
- 82 Jørgensen, A., Nielsen, J.E., Blomberg Jensen, M., Græm, N. and Rajpert-De Meyts, E. (2012) Analysis of meiosis regulators in human gonads: a sexually dimorphic spatio-temporal expression pattern suggests involvement of DMRT1 in meiotic entry. *Mol. Hum. Reprod.* **18**, 523–534 <https://doi.org/10.1093/molehr/gas030>
- 83 Shimada, R., Koike, H., Hirano, T. and Saga, Y. (2020) NANOS2 suppresses the cell cycle by repressing mTORC1 activators in embryonic male germ cells. *bioRxiv* <https://doi.org/10.1101/2020.09.23.310912>
- 84 Bowles, J., Feng, C.-W., Spiller, C., Davidson, T.-L., Jackson, A. and Koopman, P. (2010) FGF9 suppresses meiosis and promotes male germ cell fate in mice. *Dev. Cell* **19**, 440–449 <https://doi.org/10.1016/j.devcel.2010.08.010>
- 85 Menke, D.B. and Page, D.C. (2002) Sexually dimorphic gene expression in the developing mouse gonad. *Gene Expr. Patterns* **2**, 359–367 [https://doi.org/10.1016/S1567-133X\(02\)00022-4](https://doi.org/10.1016/S1567-133X(02)00022-4)
- 86 Edelsztein, N.Y., Kashimada, K., Scheingart, H.F. and Rey, R.A. (2020) CYP26B1 declines postnatally in sertoli cells independently of androgen action in the mouse testis. *Mol. Reprod. Dev.* **87**, 66–77 <https://doi.org/10.1002/mrd.23302>
- 87 Zhang, X., Gunewardena, S. and Wang, N. (2021) Nutrient restriction synergizes with retinoic acid to induce mammalian meiotic initiation in vitro. *Nat. Commun.* **12**, 1758 <https://doi.org/10.1038/s41467-021-22021-6>
- 88 Smith, H.E., Su, S.S., Neigeborn, L., Driscoll, S.E. and Mitchell, A.P. (1990) Role of IME1 expression in regulation of meiosis in *saccharomyces cerevisiae*. *Mol. Cell. Biol.* **10**, 6103–6113 <https://doi.org/10.1128/mcb.10.12.6103-6113.1990>
- 89 Tada, T., Tada, M., Hilton, K., Barton, S.C., Sado, T., Takagi, N. et al. (1998) Epigenotype switching of imprintable loci in embryonic germ cells. *Dev. Genes Evol.* **207**, 551–561 <https://doi.org/10.1007/s004270050146>
- 90 Schultz, R.M., Stein, P. and Svoboda, P. (2018) The oocyte-to-embryo transition in mouse: past, present, and future. *Biol. Reprod.* **99**, 160–174 <https://doi.org/10.1093/biolre/roy013>
- 91 Yokobayashi, S., Liang, C.-Y., Kohler, H., Nestorov, P., Liu, Z., Vidal, M. et al. (2013) PRC1 coordinates timing of sexual differentiation of female primordial germ cells. *Nature* **495**, 236–240 <https://doi.org/10.1038/nature11918>
- 92 Hill, P.W.S., Leitch, H.G., Requena, C.E., Sun, Z., Amouroux, R., Roman-Trufero, M. et al. (2018) Epigenetic reprogramming enables the transition from primordial germ cell to gonocyte. *Nature* **555**, 392–396 <https://doi.org/10.1038/nature25964>
- 93 Hajkova, P., Ancelin, K., Waldmann, T., Lacoste, N., Lange, U.C., Cesari, F. et al. (2008) Chromatin dynamics during epigenetic reprogramming in the mouse germ line. *Nature* **452**, 877–881 <https://doi.org/10.1038/nature06714>
- 94 Walfridsson, J., Khorosjutina, O., Matikainen, P., Gustafsson, C.M. and Ekwall, K. (2007) A genome-wide role for CHD remodelling factors and Nap1 in nucleosome disassembly. *EMBO J.* **26**, 2868–2879 <https://doi.org/10.1038/sj.emboj.7601728>
- 95 Ueda, J., Harada, A., Urahama, T., Machida, S., Maehara, K., Hada, M. et al. (2017) Testis-Specific histone variant H3t gene is essential for entry into spermatogenesis. *Cell Rep.* **18**, 593–600 <https://doi.org/10.1016/j.celrep.2016.12.065>
- 96 Ge, W., Chen, C., De Felici, M. and Shen, W. (2015) In vitro differentiation of germ cells from stem cells: a comparison between primordial germ cells and in vitro derived primordial germ cell-like cells. *Cell Death Dis.* **6**, e1906 <https://doi.org/10.1038/cddis.2015.265>
- 97 Hikabe, O., Hamazaki, N., Nagamatsu, G., Obata, Y., Hirao, Y., Hamada, N. et al. (2016) Reconstitution in vitro of the entire cycle of the mouse female germ line. *Nature* **539**, 299–303 <https://doi.org/10.1038/nature20104>
- 98 Hamazaki, N., Kyogoku, H., Araki, H., Miura, F., Horikawa, C., Hamada, N. et al. (2021) Reconstitution of the oocyte transcriptional network with transcription factors. *Nature* **589**, 264–269 <https://doi.org/10.1038/s41586-020-3027-9>
- 99 Hayashi, K., Ohta, H., Kurimoto, K., Aramaki, S. and Saitou, M. (2011) Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. *Cell* **146**, 519–532 <https://doi.org/10.1016/j.cell.2011.06.052>
- 100 Shimamoto, S., Nishimura, Y., Nagamatsu, G., Hamada, N., Kita, H., Hikabe, O. et al. (2019) Hypoxia induces the dormant state in oocytes through expression of *Foxo3*. *Proc. Natl Acad. Sci. U.S.A.* **116**, 12321–12326 <https://doi.org/10.1073/pnas.1817223116>
- 101 Mukaiyama, H., Iwaki, T., Idiris, A. and Takegawa, K. (2011) Processing and maturation of carboxypeptidase Y and alkaline phosphatase in schizosaccharomyces pombe. *Appl. Microbiol. Biotechnol.* **90**, 203–213 <https://doi.org/10.1007/s00253-010-3031-3>
- 102 Tsukada, M. and Ohsumi, Y. (1993) Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*. *FEBS Lett.* **333**, 169–174 [https://doi.org/10.1016/0014-5793\(93\)80398-E](https://doi.org/10.1016/0014-5793(93)80398-E)

- 103 Matsuhara, H. and Yamamoto, A. (2016) Autophagy is required for efficient meiosis progression and proper meiotic chromosome segregation in fission yeast. *Genes Cells* **21**, 65–87 <https://doi.org/10.1111/gtc.12320>
- 104 Wen, F., Guo, Y., Hu, Y., Liu, W., Wang, Q., Wang, Y. et al. (2016) Distinct temporal requirements for autophagy and the proteasome in yeast meiosis. *Autophagy* **12**, 671–688 <https://doi.org/10.1080/15548627.2016.1149659>
- 105 Barth, J.M.I., Szabad, J., Hafen, E. and Köhler, K. (2011) Autophagy in *Drosophila* ovaries is induced by starvation and is required for oogenesis. *Cell Death Differ.* **18**, 915–924 <https://doi.org/10.1038/cdd.2010.157>
- 106 Song, Z.-H., Yu, H.-Y., Wang, P., Mao, G.-K., Liu, W.-X., Li, M.-N. et al. (2015) Germ cell-specific Atg7 knockout results in primary ovarian insufficiency in female mice. *Cell Death Dis.* **6**, e1589 <https://doi.org/10.1038/cddis.2014.559>
- 107 Shang, Y., Wang, H., Jia, P., Zhao, H., Liu, C., Liu, W. et al. (2016) Autophagy regulates spermatid differentiation via degradation of PDLIM1. *Autophagy* **12**, 1575–1592 <https://doi.org/10.1080/15548627.2016.1192750>
- 108 Ferder, I.C., Fung, L., Ohguchi, Y., Zhang, X., Lassen, K.G., Capen, D. et al. (2019) Meiotic gatekeeper STRA8 suppresses autophagy by repressing Nr1d1 expression during spermatogenesis in mice. *PLoS Genet.* **15**, e1008084 <https://doi.org/10.1371/journal.pgen.1008084>
- 109 Cheng, C.Y. and Mruk, D.D. (2012) The blood-testis barrier and its implications for male contraception. *Pharmacol. Rev.* **64**, 16–64 <https://doi.org/10.1124/pr.110.002790>
- 110 Lord, T. and Nixon, B. (2020) Metabolic changes accompanying spermatogonial stem cell differentiation. *Dev. Cell* **52**, 399–411 <https://doi.org/10.1016/j.devcel.2020.01.014>
- 111 Gomes, L.R., Menck, C.F.M. and Leandro, G.S. (2017) Autophagy roles in the modulation of DNA repair pathways. *Int. J. Mol. Sci.* **18**, 2351 <https://doi.org/10.3390/ijms18112351>
- 112 Wei, Y., Lee, N.N., Pan, L., Dhakshnamoorthy, J., Sun, L.-L., Zofall, M. et al. (2021) TOR targets an RNA processing network to regulate facultative heterochromatin, developmental gene expression and cell proliferation. *Nat. Cell Biol.* **23**, 243–256 <https://doi.org/10.1038/s41556-021-00631-y>
- 113 Beauchamp, E.M. and Platanias, L.C. (2013) The evolution of the TOR pathway and its role in cancer. *Oncogene* **32**, 3923–3932 <https://doi.org/10.1038/onc.2012.567>
- 114 Zheng, X.-F. and Schreiber, S.L. (1997) Target of rapamycin proteins and their kinase activities are required for meiosis. *Proc. Natl Acad. Sci. U.S.A.* **94**, 3070–3075 <https://doi.org/10.1073/pnas.94.7.3070>
- 115 Wei, Y., Reveal, B., Reich, J., Laursen, W.J., Senger, S., Akbar, T. et al. (2014) TORC1 regulators Iml1/GATOR1 and GATOR2 control meiotic entry and oocyte development in *Drosophila*. *Proc. Natl Acad. Sci. U.S.A.* **111**, E5670–E5677 <https://doi.org/10.1073/pnas.1419156112>
- 116 Dalfó, D., Michaelson, D. and Hubbard, E.J.A. (2012) Sensory regulation of the *C. elegans* germline through TGF- β -dependent signaling in the niche. *Curr. Biol.* **22**, 712–719 <https://doi.org/10.1016/j.cub.2012.02.064>
- 117 Korta, D.Z., Tuck, S. and Hubbard, E.J.A. (2012) S6k links cell fate, cell cycle and nutrient response in *C. elegans* germline stem/progenitor cells. *Development* **139**, 859–870 <https://doi.org/10.1242/dev.074047>
- 118 Michaelson, D., Korta, D.Z., Capua, Y. and Hubbard, E.J.A. (2010) Insulin signaling promotes germline proliferation in *C. elegans*. *Development* **137**, 671–680 <https://doi.org/10.1242/dev.042523>
- 119 Suzuki, S., McCarrey, J.R. and Hermann, B.P. (2021) An mTORC1-dependent switch orchestrates the transition between mouse spermatogonial stem cells and clones of progenitor spermatogonia. *Cell Rep.* **34**, 108752 <https://doi.org/10.1016/j.celrep.2021.108752>
- 120 Sahin, P., Gungor-Ordueri, N.E. and Celik-Ozenci, C. (2018) Inhibition of mTOR pathway decreases the expression of pre-meiotic and meiotic markers throughout postnatal development and in adult testes in mice. *Andrologia* **50**, e12811 <https://doi.org/10.1111/and.12811>
- 121 Bruggeman, J.W., Koster, J., Lodder, P., Repping, S. and Hamer, G. (2018) Massive expression of germ cell-specific genes is a hallmark of cancer and a potential target for novel treatment development. *Oncogene* **37**, 5694–5700 <https://doi.org/10.1038/s41388-018-0357-2>
- 122 Sandhu, S., Salmon, L.J., Hunter, J.E., Wilson, C.L., Perkins, N.D., Hunter, N. et al. (2019) A pseudo-meiotic centrosomal function of TEX12 in cancer. *bioRxiv* <https://doi.org/10.1101/509869>
- 123 Nielsen, A.Y. and Gjerstorff, M.F. (2016) Ectopic expression of testis germ cell proteins in cancer and its potential role in genomic instability. *Int. J. Mol. Sci.* **17**, 890 <https://doi.org/10.3390/ijms17060890>
- 124 Nishikawa, H., Maeda, Y., Ishida, T., Gnjatic, S., Sato, E., Mori, F. et al. (2012) Cancer/testis antigens are novel targets of immunotherapy for adult T-cell leukemia/lymphoma. *Blood* **119**, 3097–3104 <https://doi.org/10.1182/blood-2011-09-379982>
- 125 Bline, A.P., Le Goff, A. and Allard, P. (2020) What is lost in the weismann barrier? *J. Dev. Biol.* **8**, 35 <https://doi.org/10.3390/jdb8040035>
- 126 Jeffery, C.J. (2018) Protein moonlighting: what is it, and why is it important? *Philos. Trans. R. Soc. B Biol. Sci.* **373**, 20160523 <https://doi.org/10.1098/rstb.2016.0523>
- 127 Espagne, E., Vasnier, C., Storlazzi, A., Kleckner, N.E., Silar, P., Zickler, D. et al. (2011) Sme4 coiled-coil protein mediates synaptonemal complex assembly, recombinosome relocalization, and spindle pole body morphogenesis. *Proc. Natl Acad. Sci. U.S.A.* **108**, 10614–10619 <https://doi.org/10.1073/pnas.1107272108>
- 128 De Muyt, A., Zhang, L., Piolot, T., Kleckner, N., Espagne, E. and Zickler, D. (2014) E3 ligase Hei10: a multifaceted structure-based signaling molecule with roles within and beyond meiosis. *Genes Dev.* **28**, 1111–1123 <https://doi.org/10.1101/gad.240408.114>
- 129 Ahmadiankia, N., Bagheri, M. and Fazli, M. (2019) Nutrient deprivation modulates the metastatic potential of breast cancer cells. *Rep. Biochem. Mol. Biol.* **8**, 139–146 PMID:31832437
- 130 Harachi, M., Masui, K., Okamura, Y., Tsukui, R., Mischel, P.S. and Shibata, N. (2018) mTOR complexes as a nutrient sensor for driving cancer progression. *Int. J. Mol. Sci.* **19**, 3267 <https://doi.org/10.3390/ijms19103267>
- 131 Pavlova, N.N. and Thompson, C.B. (2016) The emerging hallmarks of cancer metabolism. *Cell Metab.* **23**, 27–47 <https://doi.org/10.1016/j.cmet.2015.12.006>
- 132 Waites, G.M. and Gladwell, R.T. (1982) Physiological significance of fluid secretion in the testis and blood-testis barrier. *Physiol. Rev.* **62**, 624–671 <https://doi.org/10.1152/physrev.1982.62.2.624>
- 133 Kirmes, I., Szczurek, A., Prakash, K., Charapitsa, I., Heiser, C., Musheev, M. et al. (2015) A transient ischemic environment induces reversible compaction of chromatin. *Genome Biol.* **16**, 246 <https://doi.org/10.1186/s13059-015-0802-2>
- 134 Weisman, R. (2021) Nutrient-sensitive heterochromatinization by TOR. *Nat. Cell Biol.* **23**, 214–216 <https://doi.org/10.1038/s41556-021-00647-4>