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DOI: 10.1038/nrgXXXX [office use only]

Abstract | The germ line represents a continuous cellular link between generations and between species, but the germ cells themselves develop in a specialized, organism-specific context. The three most common animal model organisms, *C. elegans*, *Drosophila*, and mouse, display striking similarities as well as major differences in the means by which they control germ cell development. Comparison of the germ cells of these three organisms sheds light not only on universal aspects of germline regulation, but also on control of the pluripotent state *in vivo* and on the earliest steps of embryogenesis. Taking a broad perspective, we will follow the germ cells from specification in the early embryo, through gametogenesis, to fertilization, and highlight themes from the comparison of three alternative strategies for navigating the fundamental cycle of sexual reproduction.

Introduction

The germ line is the common thread connecting the past, present, and future of a species. Somatic mutations and environmental forces may have drastic effects on individual organisms, but only the information encoded in the germ line will be passed on from generation to generation. Even as genomes change and species evolve, the germ line provides a continuous link stretching back to a common ancestor. To provide this continuity, however, the cells that physically carry the genome from parent to offspring – the germ cells – must negotiate an intricate series of developmental processes within a species-specific context. Germ cells chaperone the diploid genome through embryogenesis, divide it neatly into two complete haploid genomes during meiosis, and prepare it to combine with a second haploid genome at fertilization to begin the process again.

Recent years have witnessed the emergence of technologies and approaches that have transformed germ cell biology from an essentially embryological field to one grounded in genetics and molecular biology. High-throughput sequencing now permits the characterization of expression states in small amounts of tissue, while the increasing availability of genetic reagents in mice is permitting mammalian studies to approach the

elegant understanding previously established in *Caenorhabditis elegans* and *Drosophila melanogaster*. Knowledge emerging from molecular studies of germ cell development can now be integrated with older studies that characterized these cells histologically to generate a more comprehensive understanding of this ancient and fundamental cell population. This transition is only beginning, and its implications extend beyond the field of germ cell biology. In addition to their role as carriers of information between generations, germ cells represent an *in vivo* cell population that is closely related to extensively-studied *in vitro* pluripotent stem cell models. The emerging molecular understanding of germ cell biology will lend much to the ongoing dialog about the nature and limits of pluripotency and of pluripotent stem cells. A deeper understanding of how the genome is regulated and protected in the germ cells will also inform clinical practice regarding infertility and heritable disease.

Here, we will summarize the genetic control of germ cell development in *C. elegans*, *D. melanogaster*, and mouse, underlining similarities and differences in the ways these animals arrange for the passage of their genomes from one generation to the next (Figure 1). We will discuss four tasks shared by germ cells in all three species: establishment of a germline lineage; maintenance of that lineage by global transcriptional repression; initiation of meiosis and its relationship to sex determination and gametogenesis; and preparation of the GAMETES for regulation of early zygotic processes after fertilization. We will focus on genetic regulation of these processes, leaving detailed discussion of the cell biological changes associated with migration and gametogenesis, and of the intricacies of meiosis, for other reviews.[1-4]

Specification of the germline

Germline specification in a given species is traditionally classified as occurring either by “preformation”, whereby germ cell precursors are specified by maternally deposited factors, or by induction, whereby germ cells are recruited from a multipotent embryonic cell population by signals arising from surrounding tissue.[5] Among the three organisms discussed here, worms and flies exhibit the former mechanism, and mice the latter. Importantly, while this division falls along the vertebrate-invertebrate line among these three organisms, this distribution is not generalizable. Like mice, all mammals generate

their germ cells by induction, but other vertebrates such as zebrafish and *Xenopus* set aside a GERM PLASM by preformation, while many invertebrates, including annelid worms, molluscs, and many insects, segregate their germline using inductive mechanisms.[5]

'Preformation' in worms and flies. The division between preformation and inductive germ cell specification depends on whether there is physical continuity of germ cell cytoplasm between one generation and the next. Both *C. elegans* and *D. melanogaster* maintain specific cytoplasmic complexes, referred to as germ plasm, in the one-cell embryo and in the cells that are subsequently set aside to form the germline. Germ plasm complexes are composed of RNA and protein; many of the proteins are RNA-binding factors involved in translational regulation of germ plasm mRNAs, and many of these are conserved in germ cells across multiple species (**Table 1**).[6]

In worms, the one-cell embryo (P_0) contains mRNA-protein complexes (P GRANULES) that are initially dispersed throughout the cytoplasm. During the first four embryonic divisions, the P granules segregate specifically into the cells of the P lineage. After these four divisions, P_4, the precursor of all germ cells in the adult animal, divides to form the two germline founder cells Z2 and Z3.[7] P granule components are deposited maternally and are protected from degradation specifically in the germ cell lineage.[8] Most protein components are RNA-binding proteins, including the conserved germ cell regulators GLH-1,2, 3 and 4, the P granule assembly proteins PGL-1, 2, and 3, and the meiotic regulators OMA-1 and 2.[6] Depletion or loss of mRNA or protein P granule components results in sterility, although frequently two or more redundant family members must be eliminated to achieve complete penetrance. However, mutant embryos that fail to asymmetrically segregate P granules during embryogenesis are still able to asymmetrically segregate specific germ plasm components into the germ cell lineage, and in adulthood these mutant animals are fertile and assemble P granules uniquely in the germline.[8] Thus, while germ plasm components are central to germline development and are assembled into P granules, germline-specific segregation of P granules is not required for germ cell development.

D. melanogaster oocytes contain localized mRNAs and proteins even before fertilization, poising them for germ cell segregation soon after the sperm enters the oocyte.

The POLAR_GRANULES, the equivalent of *C. elegans* P granules, localize to the posterior pole of the oocyte and guide both abdomen patterning and germ plasm assembly during embryogenesis.[9,10] Transplantation of cytoplasm from the posterior to the anterior pole of the embryo at the early cleavage stage is sufficient to induce both abdomen and germ cell precursor (pole cell) formation in the anterior.[9] The polar granule component *oskar* is necessary and sufficient for production of pole cells in the early embryo. The number of pole cells formed depends on the amount of *oskar* present, and mislocalization of *oskar* mRNA recruits other polar granule components and results in the ectopic formation of pole cells, similar to the effects of transplanted pole plasm.[11] Maternally provided pole plasm proteins recruited to the posterior pole by Oskar, such as Vasa, Tudor, and Valois, regulate the localization and translation of RNAs such as *nanos* and *polar granule component* (*pgc*, see below) that are required for primordial germ cell formation, specification and function.[10-14] In addition, the Sm proteins, RNA-binding proteins that make up the splicesome, play a splicing-independent role in specification of the germline in both *D. melanogaster* and *C. elegans*.[15-17]

Induction of the germline in mouse. In the mouse, all cells in the embryo are capable of contributing to the germline until after the blastocyst stage, indicating that there is no specific germ cell lineage predetermined by maternally inherited cytoplasmic factors. The exact time at which germline precursors are first specified is unknown; the earliest known germ cell markers, the PR- and SET-domain-containing genes *Prdm1* (also called *Blimp1*) and *Prdm14,* are expressed independently of each other in approximately 6 cells in the proximal-posterior EPIBLAST, close to the EXTRAEMBRYONIC ECTODERM, at around embryonic day 6.25 (E6.25).[18,19] These few cells are recruited from a larger population of cells competent but not destined to form the germline, identified by expression of the transmembrane protein IFITM3 (also called FRAGILIS).[20,21] Recruitment and subsequent induction of germ cell precursors are position-dependent: cells transplanted from distal regions of the epiblast to the proximal-posterior region will form germ cell precursors with approximately equal efficiency as will native proximal epiblast cells.[22] In the region of induction, an epiblast-derived WNT3 signal primes cells to respond to the diffusible signaling peptide bone morphogenetic protein 4 (BMP4), which is produced in the extraembryonic ectoderm and induces the germ cell precursor state.[23] A still-unspecified

antagonistic signal from the ANTERIOR VISCERAL ENDODERM prevents cells in the anterior epiblast from contributing to the germ cell lineage. The additional BMP family members BMP8b and BMP2, coming from the extraembryonic ectoderm, oppose the inhibitory anterior signal, setting up a gradient of inhibition and supporting induction of germ cells in the posterior epiblast.[20,24-26] During this time, the pluripotency-associated genes *Oct4*, *Sox2*, and *Nanog* are progressively restricted to the germline and become germ cell specific at E8.0.[27-29]

The means by which a limited subset of cells is initially selected from among the larger number of competent cells in the correct position of the posterior epiblast is unclear. Of the genes known to regulate germ cell identity at the time of specification, none fits the necessary criteria to serve as a germ cell selector. *Prdm14* and *Prdm1* are not required for specification.[18,19] In *Prdm1* mutants, competent epiblast cells initiate a transcriptional program similar to that of germ cell precursors (PRIMORDIAL GERM CELLS, or PGCs), but fail to progress past the early stages of specification and eventually die.[18] *Prdm14* mutants make it further in development as PGCs, but these cells, too, eventually die.[19] The early germ cell marker developmental pluripotency-associated 3 (*Dppa3*, also called *Stella*), first detected in the germ cell precursor population at E7.0, is specific to the germ cell lineage but is not required for germ cell specification, as germ cell development in *Dppa3* knockout mice is unperturbed.[30] *Lin28*, a negative regulator of the microRNA let-7, is required for *Prdm1* expression in developing germ cell precursors, and knockdown of *Lin28* results in reduced numbers of germ cells. However, *Lin28* is expressed in presumptive mesodermal cells in the proximal epiblast as well as in germ cell precursors, so it is not sufficient to specify germ cell fate.[31] Likewise, *Oct4* is required to form *Dppa3*-expressing PGCs but is expressed in cells outside the putative germ cell lineage at the time of specification.[29,32]

Despite the division between preformation and induction, fundamental similarities exist in the germ cell specification of worm, fly, and mouse. Each species sets aside a dedicated set of germ cell precursors – P_4 in *C. elegans*, pole cells in *D. melanogaster*, and *Prdm1*$^{+}$/*Prdm14*$^{+}$precursor cells in mouse – during a relatively short window during development. A major difference between species lies in the time at which the window of competence opens and closes: in worms, polarization of the zygote at fertilization establishes the germ cell lineage; in flies, the future germ cell cytoplasm is determined in

the oocyte even before fertilization; and in mice, specification occurs only after embryogenesis has begun.

The role of transcriptional repression in maintenance of germ cell identity

Following specification, germ cell precursors in all three species are dependent on stringent regulation of gene expression to prevent initiation of somatic transcriptional programs and to convert specification signals into long-lasting developmental states. Strict transcriptional control is particularly important in the interval immediately following specification, during which the embryo begins GASTRULATION and germ cells are inundated with potent inductive signals involved in somatic tissue specification. Germ cells in mouse and *D. melanogaster* (but not in *C. elegans*) also spend this period migrating long distances through the embryo, traveling from their site of specification to the region of the developing gonad (reviewed in ref. 1).[1] They must resist an ever-changing set of external cues, while retaining their relatively undifferentiated state.[1,33,34] To maintain the germ cell state, all three organisms invoke chromatin-based repression mechanisms, and *C. elegans* and *D. melanogaster* also employ a strategy of universal direct transcriptional repression through inhibition of RNA polymerase II (RNAPII).

Regulation of RNA polymerase II. *C. elegans* germ cell precursors are transcriptionally silent from the time of fertilization until approximately the 100-cell stage, after gastrulation begins.[35] A maternally-inherited P granule protein, PIE-1, is required for this transcriptional silencing: in embryos derived from *pie-1* mutants, zygotic transcripts are aberrantly present in the embryonic germ cell precursors, and the germline does not develop.[35,36] Ordinarily, the kinase complex Positive Transcription Elongation Factor b (P-TEFb) recognizes and binds to the RNAPII C-terminal domain (CTD), phosphorylating serine 2 of the CTD to drive transcriptional elongation (**Figure 2a**). PIE-1 has a C-terminal tail sequence similar to that of RNAPII and competes for P-TEFb. When PIE-1 is present, P-TEFb is thought to be recruited away from RNAPII, preventing phosphorylation of Ser2 and inhibiting transcriptional elongation (**Figure 2b**).[37,38] In addition, PIE-1 uses a different but still undefined mechanism to prevent phosphorylation of serine 5 of the CTD, thereby inhibiting PREINITIATION COMPLEX formation and transcriptional initiation.[39]

Transcription is repressed in the early *D. melanogaster* germline by a mechanistically similar but independently derived mechanism. As in *C. elegans*, pole cells are transcriptionally inactive until mid-embryogenesis, and a maternal-effect gene, *polar granule component* (*pgc*), is required to maintain transcriptional silencing.[40] In embryos derived from females lacking a functional *pgc* gene, zygotic transcription is not repressed in the pole cells and they begin to degenerate at about the time zygotic expression begins in the surrounding soma, ultimately resulting in loss of the germline.[41,42] Like PIE-1, Pgc protein interacts with P-TEFb, inhibiting its ability to bind to and phosphorylate the C-terminal tail of RNAPII (**Figure 2c**).[43] In mice, levels of phosphorylation on serines 2 and 5 of the RNAPII CTD are also reduced in PGCs, corresponding to a reduction in RNAPII-dependent transcription, although unlike their counterparts in worms and flies, these cells retain some transcriptional activity. Whether the reduced Ser2 and Ser5 phosphorylation seen in murine PGCs also occurs via global inhibition of P-TEFb remains unknown.[44]

Regulation of chromatin. Developing germ cells in all three species also employ chromatin-based repression. In *D. melanogaster* and *C. elegans*, repressive chromatin configurations take over from PIE-1 or Pgc-based global transcriptional inhibition around the time of gastrulation, when PIE-1 or Pgc protein levels decline.[45] The chromatin of the *C. elegans* germline founder cells, Z2 and Z3, is more condensed than that of the surrounding somatic cells. Z2 and Z3 have lower levels of the activating histone modifications di- and tri-methyl lysine 4 on histone H3 (H3K4me2 and H3K4me3) and acetylated lysine 8 on histone H4 (H4K8ac), and slightly higher levels of the repressive modification trimethyl lysine 27 on histone H3 (H3K27me3).[45,46] A complex of maternal-effect proteins including the POLYCOMB group orthologs MES-2 and MES-6 is required for deposition of the repressive H3K27me3 mark in germ cells; individuals lacking any of these complex components have offspring that fail to develop a germline.[46-48] Likewise, loss of the LSD1 histone demethylase homolog *spr-5* results in progressive failure to demethylate H3K4, impaired transcriptional repression, and cumulative sterility over multiple generations, while absence of the *nanos* homologs *nos-1* and *nos-2* causes premature re-accumulation of H3K4me2/3 in the embryonic germline and corresponding germline failure and sterility.[45,49] In *D. melanogaster*, germ cell precursors lacking *nanos* or the *spr-5*/LSD1 histone demethylase homolog Suppressor of variegation 3-3 (SU(VAR)3-3) also exhibit

elevated levels of H3K4me2 and fail to appropriately maintain repression of developmental genes.[45,50]

In mice, invocation of a repressive chromatin configuration is among the earliest events in the germ cell lineage. PRDM1, the early PGC marker, is required for repression of somatic developmental regulators.[51] PRDM1 associates with the arginine methyltransferase PRMT5, which mediates dimethylation of arginine 3 on histone H2A, a repressive modification, and association with PRMT5 is required for at least some instances of PRDM1-mediated transcriptional repression.[52] In *Prdm1* mutants, germ-cell-like precursors are specified in approximately normal numbers, but these cells inappropriately express Hox genes and other developmental transcription factors, fail to proliferate, and eventually die.[18] *Prdm1* mutants do maintain expression of actively transcribed germ cell genes, including *Oct4* and *Sox2*.[51] Levels of the repressive H3K27me3 histone modification increase drastically in migrating mouse germ cells at around E9.0, following loss of Ser2 and Ser5 phosphorylation on RNAPII, and remain high as RNAPII phosphorylation reaccumulates. Increased deposition of this repressive chromatin mark may serve to maintain a transcriptionally repressed state in the PGCs, although a direct requirement for elevated levels of H3K27me3 in maintaining transcriptional repression in this context has not been demonstrated.[44]

Meiotic initiation and germ cell sex determination

Entry into the first stages of gametogenesis requires exit from the transcriptionally sheltered state enjoyed by early germ cells, followed by the decision to initiate a sex-specific (egg or sperm) differentiation pathway and preparation for meiotic cell division. Sex determination and meiotic initiation are regulated by many of the same genes and are sometimes temporally intertwined; the extent to which they are mechanistically separable is an active area of investigation.

Meiotic initiation and sex determination in C. elegans. In *C. elegans*, the two germline precursor cells Z2 and Z3 begin to divide mitotically towards the middle of the first larval stage, producing a pool of GERMLINE STEM CELLS (GSCs), and the first GSCs enter meiosis as the third larval stage (L3) begins.[53] The decision to enter meiosis is determined by distance from a somatic "NICHE", the DISTAL TIP CELL. The distal tip cell produces a Delta-

like signal, LAG-2, that inhibits meiotic entry and promotes mitotic proliferation (**Figure 3a**). LAG-2 signals through GLP-1, a Notch receptor on the GSCs.[54,55] In *glp-1* mutants, GSCs enter meiosis in early L3 and fail to maintain a proliferative germ cell pool; conversely, *glp-1* gain-of-function mutations cause a tumor-like expansion of the germline.[54,56] Meiotic entry is also regulated by a set of RNA-binding proteins, the PUF (Pumilio and FBF) family proteins FBF-1 and FBF-2. These proteins inhibit meiotic entry by preventing translation of the meiosis-promoting genes GLD-1, a translational repressor, and GLD-3, a translational activator, that act along with the poly(A) polymerase GLD-2 and the *nanos* homolog NOS-3 to coordinate meiotic initiation.[57-59] GLD-3 and NOS-3 inhibit FBF-1 and FBF-2 function, maintaining the meiotic state once it has been initiated.[60,61]

C. elegans hermaphrodites produce male and female gametes sequentially: the first few rounds of meiosis produce SPERMATOCYTES, and the rest of the GSCs then commit to OOCYTE production for the remainder of the animal's life. As a result, the timing of meiotic entry in the *C. elegans* life cycle is intimately tied to gamete sex determination. FOG-1, a cytoplasmic poly-A element binding (CPEB) protein, and FOG-3, a Tob/BTG transcriptional/translational regulator, promote SPERMATOGENESIS; they are inhibited by the FBF proteins and by TRA-1, a conserved transcription factor that promotes female/hermaphrodite specification in somatic tissues (reviewed in refs 62 and 63).[62-68] TRA-1 is inhibited by FEM-3, which acts along with the additional factors FEM-1 and FEM-2 to push the germ cells toward continued spermatogenesis.[69,70] FEM-3 is opposed by the RNA-binding protein DAZ-1, which promotes the switch to OOGENESIS.[71] DAZ-1 also promotes transcription of *fbf-1* and *fbf-2* mRNAs, and the FBF-1 and FBF-2 proteins repress *fem-3* translation; as a result, the FBF proteins act both to inhibit meiosis and to promote entry into oogenesis.[71,72]

Meiotic initiation and sex determination in D. melanogaster. Pole cells are carried from the posterior tip of the embryo into the interior during gastrulation, and then migrate to the developing somatic gonad.[73] During this time, they are transcriptionally quiescent and arrested in mitotic G_2.[74] Transcription of a few genes begins just before the pole cells enter the nascent gonad; a further increase in transcription coincides with resumption of mitotic cell cycles following gonad entry.[75] The transition to gametogenesis

in flies therefore represents the transition from a developmental program largely under maternal control to a *bona fide* embryonic program.

In contrast to *C. elegans*, *D. melanogaster* sex determination and meiotic entry are temporally separated. Sex determination occurs in the embryo. Male and female germ cells alike initiate sex-specific differentiation soon after entering the gonad progenitor, under the guidance of both somatic cues and germ cell autonomous signals. *Sex lethal (Sxl)*, a major determinant of somatic sexual identity, is transiently expressed in female but not male PGCs and is cell-autonomously required for female germ cell identity.[76] Additional factors, including *ovarian tumor* (OTU) and the transcription factor OVO-B, are also expressed in a female-specific manner in germ cells and act to promote a female identity.[77-79] Different cell-intrinsic signals, downstream of a JAK-STAT signaling pathway, are required for male germ cell identity but remain largely unidentified.[80] In both sexes, meiosis begins much later, around the time of pupation, with mature sperm present by ECLOSION and mature eggs present soon afterward.[81,82]

Meiotic initiation and progression are orchestrated by gonad structure. Signaling from a somatic niche maintains mitotic precursors: in females, CAP CELLS produce the BMP signals *decapentaplegic* (Dpp) and *glass bottom boat* (Gbb), and in males the HUB produces the cytokine-like ligand *unpaired* (Upd) (**Figure 3b**). The RNA binding proteins Pumilio and Nanos are cell-intrinsically required in GSCs to maintain proliferation and prevent differentiation.[83,84] GSCs divide to produce one replacement GSC and one CYSTOBLAST. The cystoblast loses its physical attachment to the somatic niche, exposing it to reduced levels of niche-derived self-renewal signals and allowing it to embark on the path to meiosis.[85-87] Both sexes require *bag-of-marbles (bam)* and *benign germ cell neoplasm (bgcn)* to promote the transition from GSC to cystoblast, and to orchestrate the transition from mitosis to meiosis. *Bam* and *Bcgn* act together as translational regulators; loss of function of either gene results in tumor-like overgrowth of mitotic GSCs (**Figure 3b**).[88-90] In both sexes, cystoblasts divide mitotically four times with incomplete cytokinesis to generate 16 linked daughter cells, collectively called a cyst.[91,92] In males, all cyst cells enter meiosis and mature as sperm, while in females only some cells within a cyst initiate expression of meiotic genes, and only one of these is chosen to complete development as an oocyte; the remaining 15 cells, called nurse cells, contribute cytoplasmic resources to

the oocyte and eventually die when oogenesis is complete.[91] Mutations in the genes *bicaudal D*, *egalitarian*, and *orb* all perturb oocyte selection and result in formation of cysts with no oocyte.[93]

Meiotic initation and sex determination in mouse. Murine PGCs travel from their site of specification in the proximal epiblast through the developing gut, and enter the gonadal precursor during mid-embryogenesis. Like migrating *D. melanogaster* pole cells, they have low transcriptional activity, but unlike pole cells they continue to divide mitotically throughout this interval. The PGCs begin to reach the developing gonad around E10.5.[94] Over the next 48 hours, PGCs of both sexes rapidly lose DNA cytosine methylation and initiate expression of a handful of shared transitional genes.[95-98] During this transitional period, they undergo LICENSING, meaning that they exit their pluripotent, migratory state and acquire competence to initiate sexual differentiation and enter meiosis (**Figure 3c**). Licensing depends on the RNA-binding protein Deleted in Azoospermia-Like (DAZL): *Dazl* mutants fail to express markers of male or female differentiation and fail to initiate meiosis.[99,100] Subsequently, female germ cells enter meiotic PROPHASE (**Figure 4a**) and begin to differentiate as oocytes, while male germ cells enter G_1/G_0 arrest until after birth.[101] As in *C. elegans*, the signals that trigger meiotic entry in males and females are similar but temporally displaced (**Figure 3c**): presumptive oocytes in mice receive these signals in the embryo, whereas spermatogenic precursors are sheltered from them until puberty. Meiotic entry in both sexes requires the HLH protein Stimulated by Retinoic Acid gene 8 (STRA8).[102,103] In females, *Stra8* is expressed in germ cells in response to retinoic acid (RA) beginning at E12.5-13.5, and meiotic prophase begins soon afterward.[104] In prenatal males, the cytochrome P450 enzyme CYP26B1 degrades retinoic acid, preventing induction of *Stra8*.[105,106] *Nanos2* is specifically expressed in fetal male germ cells and also functions as a meiotic inhibitor.[107,108] Postnatally, *Cyp26b1* is repressed in male gonads, and male spermatogenic precursors gain the ability to respond to retinoic acid and to express *Stra8*, and they begin to initiate meiosis in regular waves.[103,105,106] Germ cell sex determination and meiotic entry are therefore temporally coordinated in mammals and are frequently conflated, with initiation of meiosis at E13.5 considered equivalent to female specification, although transcriptional profiling of male and female germ cells at E12.5 points to a moderate amount of sex-specific transcription a day prior to female meiotic

initiation.[109] The question of whether meiotic initiation and specification of female sex in fetal germ cells are identical processes, or are simply closely related, remains unresolved.

Preparation for embryogenesis

Mature gametes ultimately acquire the physical capabilities required to find each other, unite, and form a new diploid genome, as well as to direct the first stages of embryogenesis. Developing sperm complete meiosis, condense their chromatin, divest themselves of most of their cytoplasm, and generate a specialized motility apparatus. Developing oocytes, in contrast, carefully regulate meiotic progression, pausing at specific times to coordinate the completion of meiosis with fertilization. They also maintain high transcriptional activity, expanding their cytoplasm as much as 500 fold as they accumulate mRNAs and proteins to be used by the embryo during early development.[110]

***Preparation for embryogenesis in* C. elegans.** *C. elegans* spermatogenic precursors complete meiosis during the last larval stage. They bud off a remnant RESIDUAL BODY, leaving behind their cytoplasm, and they acquire a crawling mobility that depends on the cytoskeletal component MSP (major sperm protein).[111,112] Although spermatid nuclei condense as in other species, high levels of the histone variant H3.3 and lower levels of the canonical H3.1 are retained in mature sperm, as are patterns of H3K4 methylation established earlier in gametogenesis; some of this chromatin configuration is transferred to the zygote following fertilization.[113-115] After the last larval stage, mature sperm are stored in the spermatheca and all remaining germ cell precursors transition to oogenesis. Developing oocytes initiate meiosis and then arrest at the DIAKINESIS step of meiosis I, during which they grow in size and accumulate maternal RNAs and proteins required for early embryogenesis (**Figure 4b)**. Several genes, including *gld-1* and *daz-1*, are required for progression to this point; *daz-1* mutant oocytes arrest at PACHYTENE of meiosis I and eventually undergo apoptosis, whereas *gld-1* mutant oocytes exit meiosis and reenter the mitotic cell cycle.[116,117] Wild-type oocytes are released from arrest in prophase I by MSP, which acts hormonally as oocytes approach the spermatheca to trigger ovulation and progression into anaphase of meiosis I.[114] After the oocyte enters the spermatheca, fertilization allows completion of meiosis.[118] The site of sperm entry becomes the posterior

pole of the embryo, making fertilization the first polarizing signal in *C. elegans* embryogenesis.[119]

***Preparation for embryogenesis in* D. melanogaster.** *D. melanogaster* spermatocytes complete meiosis, condense their nuclei, replace histones with PROTAMINES, eliminate much of their cytoplasm, and produce a long flagellum. *boule*, a homolog of *C. elegans daz-1* and mouse *Dazl*, is required for completion of meiosis in sperm and for aspects of SPERMIOGENESIS.[120] Meanwhile, developing *D. melanogaster* oocytes prepare their cytoplasm for exquisite control of the initial stages of embryogenesis. The oocyte arrests at diplotene of meiosis I and remains transcriptionally active as it passes through the ovary; during this time, it is actively loaded with mRNAs and proteins synthesized by nurse cells (**Figure 4c**).[2] It is polarized along both the dorsal-ventral and anterior-posterior axes by signaling from overlying follicle cells through the EGFR ligand *gurken*. This polarization positions *bicoid* and *oskar* mRNA gradients for translation following fertilization, which will establish the embryonic A-P axis and permit rapid progression through early stages of embryogenesis following fertilization.[121] Toward the end of oogenesis, unknown signals induce progression from arrest at diplotene of prophase I to metaphase I, where a second arrest occurs. Once the oocyte passes through the oviduct, rehydration and pressure trigger passage through the last stages of meiotic division; in contrast to *C. elegans* and mouse, fertilization is not required for completion of meiosis in the *D. melanogaster* oocyte.[2] After fertilization, the paternal PRONUCLEUS rapidly de-compacts and accumulates the histone variant H3.3 as protamines are removed. If this replacement does not occur, the embryo does not survive, indicating that the rapid re-packaging of the male pronucleus is crucial to early embryogenesis.[122]

Preparation for embryogenesis in mouse. In mice, as in flies, spermatogenic cells carry out meiosis, condense their nuclei and replace histones with protamines, eliminate cytoplasm, and generate a flagellum. These processes occur exclusively during postnatal life, after male spermatogenic precursors emerge from G_1/G_0 mitotic arrest. Oocytes arrest in diplotene of meiosis I during embryogenesis and remain transcriptionally active, generating a store of proteins and mRNAs to guide early development of the zygote (**Figure 4d**). In sexually mature mice, hormonally-triggered ovulation allows the oocyte to proceed from prophase I to metaphase II, with concurrent pausing of transcription and

reduction of translation.[110] At fertilization, the egg completes meiosis, and the female pronucleus remains in a repressed state. In contrast, protamines in the male pronucleus are rapidly replaced by highly acetylated histones, producing an open chromatin state.[123] As in worms and flies, the male pronucleus preferentially incorporates the histone variant H3.3. While the female pronucleus remains transcriptionally silent, the decondensed male pronucleus produces a few transcripts in the unicellular zygote.[123] Stockpiled maternal mRNA and proteins are absolutely required at fertilization and for the first cell division; they are then actively degraded during the initial cleavage stages of embryogenesis as zygotic transcription takes over.[124,125]

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Perspectives

Every normally developing organism executes repeated mitotic cell divisions with remarkable elegance and accuracy. In contrast, the convergence of two cells, two nuclei, and two genomes into one requires a lifetime of preparation. The study of features that help to ensure the reliability and reproducibility of this process will advance our understanding of the basic mechanisms of development and inheritance, and may bring about important clinical applications.

Regulation of expression at both the transcriptional and translational levels is central to germ cell development, and may help to maintain a flexible cell state (see **Table 1)**. Posttranscriptional control in the germline operates through several mechanisms, including but not limited to recruitment of mRNAs to the ribosome, modulation of poly(A) tail length and transcript stability, restriction of translation to specific regions within the cell, and assembly of RNAs and proteins into cytoplasmic granules.[126] A single germline RNA may be subject to several of these regulatory strategies. Conversely, many RNA-binding proteins play multiple roles over the course of germ cell development, complicating the task of clearly defining a specific role for each of these proteins at a given developmental stage. The function of many of these RNA-binding factors remains unknown, and the reasons for the enrichment of this class of proteins in the germline are currently speculative. Progress in this area will require refined approaches to the unbiased study of cell-wide RNA-protein interactions, and the ability to do so in small cell populations. Recent inroads into this area

in *C. elegans* will guide the way for similar studies in other organisms.[127,128] Small RNAs, including miRNAs and piRNAs, likely play an additional role in maintaining the balance between repression and flexible gene expression in the germline (reviewed in ref. 129).[129] Similarly, histone variants and chromatin regulators specific to the germline abound, but their functions are largely unknown.[130] Molecular characterization of the epigenetic state of the germline will be necessary to understand recent reports invoking "epigenetic" inheritance (**Box 2**).

Another commonality among species is the maintenance of a proliferative pool of germline stem cells that persists through adulthood. Each of the three species described here allocates this proliferative pool differently **(Figure 1)**: in hermaphrodite worms, sperm production is restricted in time, and proliferative germline cells in the adult become oocytes; in flies, both male and female germlines maintain stem-cell-like precursors; and in mice, oocyte production is time-limited and sperm are continuously produced in adult males. In all three organisms, these proliferative pools are maintained in response to signaling from a somatic niche.[81,131-133] Because of these features, and because they retain the potential to contribute to all tissues in the embryo, germ cells are often conceptually tied to stem cells; however, the relationship between germ cells and stem cells is complex. One notable difference is that germ cells are not pluripotent in their natural setting: they are unipotent cells capable of differentiating into gametes. Only after fertilization are they capable of forming an entirely new individual. Nevertheless, there are important parallels between germ cells and pluripotent stem cells: germ cells are the only cells in the embryo that will contribute to a totipotent zygote during the natural life cycle; in mammals, primordial germ cells express several stem-cell-associated transcription factors not expressed in any other tissues, including *Oct4* and *Sox2*; and early mammalian embryonic germ cells are capable of generating pluripotent stem cells (embryonic germ, or EG, cells) in culture.[28,29,134] PGCs can also give rise to tumors, called teratomas, that produce tissues from all three germ layers, a defining trait of pluripotency.[135] Comparison of germ cells and stem cells therefore promises to improve our understanding of the nature and boundaries of pluripotency and totipotency, by providing an *in vivo* reference point for *in vitro* studies. New molecular markers for subpopulations within adult germ cell pools promise to provide insights into the balance between pluripotency and differentiation in these cells (**Box 3**).

The possibility of generating functional germ cells *in vitro*, especially from patient-derived induced pluripotent stem (iPS) cells, is of interest to the medical community as a means of addressing infertility. Only very recently have *in vitro*-derived sperm been shown to produce healthy, fertile offspring in the mouse.[136-139] To do so, spermatogenic-like cells were generated from ES and iPS cells by way of an intermediate state, called an EPIBLAST-LIKE STEM CELL (EpiSC), and then transplanted into the testes of germ-cell-depleted adult mice.[138] The necessity of proceeding through a developmentally relevant intermediate highlights the importance of understanding *in vivo* regulatory mechanisms in order to derive the most benefit from *in vitro* techniques. Functional oocytes, with their more highly regulated meiotic cycle, massive cytoplasmic growth, and requirement for a fetal context for meiotic initiation, have not yet been derived *in vitro*.[137]

The germ cells are unique in their ability to undergo meiosis and to negotiate the amalgamation of two haploid genomes into one. A molecular understanding of the genetic systems underlying germline development is beginning to emerge, and increasing knowledge in this area promises to carry the field of germ cell biology forward in new and unanticipated directions, and to open the door to important medical applications.

Box 1 | **Transgenerational epigenetic inheritance**

Several recent, high-profile studies have called attention to transgenerational epigenetic inheritance in *C. elegans*, *D. melanogaster*, and mammals. In these systems, traits induced by the environment and not directly encoded in the genome (that is, epigenetic) are passed from the exposed parent to the F1 and sometimes later generations.

In worms, mutations in the histone demethylase gene, *spr-5* (an LSD1/KDM1 homolog), cause a weak sterility phenotype that is passed on and becomes progressively more severe in subsequent generations, although the *spr-5* coding and promoter DNA sequence does not change.[49] Meanwhile, mutations in three genes encoding a histone methylase complex, *ash-2*, *wdr-5*, and *set-2*, increase longevity through several generations of offspring, even when only the founding parental animal carries the mutant

allele.[140] In *D. melanogaster*, an osmotic or heat stress stimulus can disrupt the activity of the transcription factor ATF-2 and deposition of the repressive histone modification H3K9me2, and this disruption can be transmitted to the next generation in a non-Mendelian manner.[141] In mice, F1 offspring of males fed a low-protein diet exhibit altered liver gene expression profiles, and F1 female offspring of male rats fed a high-fat diet have impaired glucose tolerance and insulin secretion despite being indistinguishable by genotype.[142,143] One historical cohort study in humans has also suggested a transgenerational effect of paternal nutrition on the metabolic status of children and grandchildren.[144,145]

These effects are intriguing, but a mechanism for their transmission between generations is often lacking from these studies. To be validated, they will require a germ-cell-based mechanism for transmission, and understanding of epigenetic regulation and genome organization throughout germ cell development will provide insights into such a mechanism. For example, the white-spotting phenotype of the *Kit*^{tm1Alf} mutant in mice was found to be transmitted by abnormally retained mRNAs in sperm, providing a mechanistic basis for this case of non-Mendelian inheritance.[146] However, current understanding of the regulation of the germ cell epigenome is insufficient to provide true insight into the means by which the effects described in many of these studies might be mediated. A deeper understanding of how DNA methylation, histone modifications, and other aspects of chromatin state are regulated in the developing germline of each of these model organisms is required to fully understand the meaning of these effects.

Box 2 | **Spermatogonial stem cells in mammals**

In male mammals, germ cells enter G_1/G_0 cell cycle arrest embryonically. After birth, they resume mitotic proliferation, and a subset of cells begins progression along a defined differentiation pathway leading to meiosis and spermiogenesis. This process produces sperm cyclically throughout adult life. The ability to recruit new spermatogenic cells throughout life implies the presence of a self-renewing pool of precursor cells, referred to as the spermatogonial stem cells (SSCs).[147,148] The presence of SSCs in adult testes has been established by transplant experiments in mice, in which labeled testicular cells are transferred to a recipient testis that has been depleted of germ cells, and are able to

reestablish ongoing spermatogenesis. However, the precise identity of the SSCs within the seminiferous tubule, and the means by which they regulate the decision to self renew or to proceed on a path toward meiosis, are a matter of lively debate.

Spermatogenic precursor cells in the testes have been classified histologically and by a limited set of marker genes, and the path along which they progress from mitotic precursor to haploid spermatid has been well mapped (see figure below for a schematic representation). Single, mitotic precursor cells, called A_{single} (A_s) spermatogonia, divide with incomplete cytokinesis to become a joined pair of cells, called A_{paired} (A_p). These pairs then divide synchronously between one and three times to become linearly conjoined chains of 4 to 16 cells, called $A_{aligned}$ (A_{al}). Following the A_{al} stage, spermatogonia are considered to be "differentiating" and pass through A_1, A_2, A_3, A_4, intermediate, B, and preleptotene stages before entering the leptotene phase of meiosis I.[147,148]

Until recently, it was assumed that A_s spermatogonia constituted the SSC pool. However, studies over the last 10 years have shown that this assumption is, at best, oversimplified. First, the testis-wide pool of A_s cells is heterogeneous, with some subsets of A_s expressing markers associated with differentiating spermatogonia and some expressing markers thought to be associated with self-renewal.[149] Long term lineage-tracing experiments indicate that there are more A_s cells than required to maintain a testis-wide pool of precursor cells.[150] In contrast, there are not enough A_s cells to provide the numbers of A_{pr} cells found in the seminiferous tubule at any given time point.[151] In partial explanation, it now appears that A_{pr} pairs, and possibly longer A_{al} chains, can break up into A_s type cells with the potential to function as SSCs.[152] One remaining question is whether these breakages occur only during periods of insult and germ cell depletion, when they have primarily been observed, or in steady-state as well.

Recent studies have begun to delineate the molecular characteristics of A_s, A_{pr}, and other spermatogonial cells, and this genetic knowledge will be enormously helpful in isolating and studying putative SSC populations. For example, the recently identified inhibitor of DNA binding 4 (ID4), a transcriptional repressor, is expressed in a subpopulation of A_s cells and is required to maintain a pool of undifferentiated spermatogonia during adulthood.[153] Therefore, ID4 is a good candidate marker for the SSCs in adult testes.

Figure legends

Figure 1 | **Summary of germ cell development in *C. elegans*, *D. melanogaster*, and mouse.**
a | Germ cell development in *C elegans, D. melanogaster* and mouse. Timescales are aligned by developmental stage rather than chronological time. Primordial germ cells (PGCs) prior to initiation of gametogenesis are shown in purple. Differentiating female gametes are shown in pink and male gametes in blue. In mouse, the germline is specified from the epiblast by inductive signals from the surrounding tissue. The PGCs divide mitotically throughout their early development. During gastrulation, they migrate to the gonad, where they gain the ability to enter meiosis and to undergo sex-specific differentiation. Female gametes initiate meiosis before birth, then enter meiotic arrest until ovulation, and male gametes initially enter G_0/G_1 arrest, beginning meiotic divisions only after birth. In *D. melanogaster*, specialized germ plasm is set aside during oogenesis and the germ cell precursors (pole cells) cellularize soon after fertilization. The pole cells migrate internally during gastrulation. After they reach the gonad, they divide mitotically and acquire sex-specific features. Meiosis begins in both sexes around the time of eclosion. In *C. elegans*, the first germline precursor is specified by the site of sperm entry at fertilization, and the two germline founder cells, Z2 and Z3, are derived from this precursor after five cell divisions. Z2 and Z3 begin to divide mitotically during the first larval stage. Meiosis begins during the third larval stage and initially produces sperm; after the end of the fourth larval stage, meiotic divisions generate oocytes. **b** | Conceptual overlay of the germ cell cycle in each organism with major gene regulatory and cell cycle events. Many of the same steps occur in all three organisms, but the timing relative to other aspects of development differs. In *Drosophila*, germline precursors are set aside and maternal control of the germline begins during oogenesis, overlapping completion of the previous cycle of germline development.

Figure 2 | **Mechanism of transcriptional repression by PIE-1 and Pgc. a** | Promotion of transcription initiation and elongation by the P-TEFb complex. P-TEFb (green rectangle)binds to the C-terminal tail of RNAPII and the Cdk9 subunit phosphorylates serine 2 (green circle) of the tail, allowing transcriptional elongation. **b** | Proposed mechanism for inhibition by PIE-1 in *C. elegans*. PIE-1 (red rectangle) has a C-terminal tail sequence similar to that of RNAPII and competes for P-TEFb, thereby inhibiting P-TEFb binding to RNAPII and phosphorylation of serines 2 and 5. The PIE-1 C-terminal tail contains alanines (orange circles) instead of serines at the Cdk9 phosphorylation sites and cannot be phosphorylated, potentially preventing dissociation of PTEFb from PIE-1 and sequestering it away from RNAPII. **c** | Inhibition by Pgc in *Drosophila*. Pgc interacts with both the Cdk9 and CycT1 components of P-TEFb and sequesters the complex away from RNAPII. It is not known whether it acts by a competition mechanism similar to PIE-1. P-TEFb, positive transcription elongation factor b; RNAPII, RNA polymerase II

Figure 3 | **Genetic pathways controlling germ cell sex determination and meiotic entry.** Diagrams show central aspects of each regulatory pathway that are discussed in the main text but do not include all genes implicated in these pathways. Genes in yellow boxes are involved in meiotic entry and genes in blue boxes are involved in sex determination. Dotted lines indicate diffusible extracellular signals that weaken when distal to the signal source. **a** | In *C. elegans*, a Notch-Delta signaling pathway promotes mitotic proliferation and GSC self-renewal, and entry into meiosis is initiated when GSCs migrate far enough away from source of LAG-2 signal. Exit from self-renewal and entry into meiosis at the last (L4) larval stage leads to male (sperm) differentiation, while the same process during adulthood results in female gametes (oocytes). The *daz-1* and *tra-1* genes are both required for the spermatogenesis-to-oogenesis switch. **b** | In *Drosophila*, the sex of the germ cells is determined early in development and specifies the signals that promote self-renewal in GSCs: that is, BMP signals in female (Dpp and Gbb) and signaling through the ligand Upd in male. The transition to meiosis occurs when the cells leave the somatic

niche. In both sexes, *bam* and *bgcn* promote cystoblast differentiation and meiotic entry. **c** | In mice, *Dazl* makes germ cells competent to enter both sex differentiation and meiotic pathways. Sex specification is determined by signals from somatic cells in the gonad, while induction of *Stra8* by RA promotes meiotic initiation. Female germ cells are able to respond to RA in the embryo, while male germ cells are prevented from doing so by CYP26B1 and by Nanos2. In adult males, DMRT1 promotes mitotic self-renewal.[154] Sohlh1 and Sohlh2 are representative effector genes involved in differentiation of both oocytes and spermatocytes.[155-157] GSC, germline stem cell; RA, retinoic acid.

Figure 4 | **Meiotic arrest in the developing oocytes. a** | Overview of the stages of meiotic prophase. In leptotene, chromosomes begin to condense; in zygotene, pairing between homologous chromosomes occurs and the synaptonemal complex holding them together is formed; in pachytene, homologous recombination ("crossing over") begins; in diplotene, the synaptonemal complex breaks down and homologous chromosomes are held together only at sites of recombination; and in diakinesis, the meiotic spindle begins to form and chromosomes condense further in anticipation of the first meiotic division. In all three species, the first meiotic arrest occurs during a specific step in meiotic prophase. **b** | Meiotic arrest in *C. elegans.* Oocytes, shown in light blue, arrest in diakinesis and are released by the hormonal signal major sperm protein (MSP). They then arrest briefly in anaphase I, but enter the spermatheca quickly afterward, are fertilized, and complete meiotic division. **c** | Meiotic arrest in *Drosophila*. The cyst consists of the 16 cells produced after four mitotic divisions of the founding precursor cell. One of these cells becomes the oocyte, while the remaining 15 are supporting nurse cells. The first meiotic arrest occurs in diplotene, and the oocyte remains arrested at this stage for much of oogenesis. Just before ovulation, it is released by an unknown signal and arrests again at metaphase I. This second arrest is relieved by rehydration and mechanical pressure during ovulation. **d** | Meiotic arrest in mouse. The first arrest occurs in diplotene during embryogenesis and lasts until after puberty, when a hormonal surge during each menstrual cycle triggers ovulation and ovulated oocytes reenter the meiotic cycle. During the first meiotic arrest, the oocyte is surrounded by supporting cells organized into a follicle. A few of these cells remain with the oocyte immediately after ovulation. Following ovulation, oocytes arrest at metaphase II, and this second arrest is released by fertilization.

Table 1 | **Selected RNA binding proteins involved in germ cell development, and their homologs.***

C. elegans		*Drosophila*		Mouse	
Name	**Role**	**Name**	**Role**	**Name**	**Role**
GLH-1 GLH-2 GLH-3 GLH-4	P granule component	Vasa	Polar granule component	DDX4 (also called MVH)	Progression through meiosis (male only)[96] piRNA expression[158]
PGL-1 PGL-2 PGL-3	P granule assembly				
OMA-1 OMA-2	Meiotic regulation				
		Oskar	Pole cell assembly[11]		
NOS-1, NOS-2, NOS-3	Transcriptional repression[45] meiotic initiation[159] sperm-oocyte switch (NOS-3)[61]	Nanos	Transcriptional & translational repression[45,160]	NANOS2 NANOS3	Inhibition of meiosis in male embryo[108] Maintenance of migrating PGCs[107]
FBF-1/2	Inhibition of meiotic entry	Pumilio	Self-renewal, inhibition of meiotic entry[161]	PUM2	Expressed in germ cells; mutant has small testes but is fertile[162]
GLD-1, GLD-3	Meiotic initiation, progression through meiosis				
FOG-1	Promotion of spermatogeneisis	Orb	In females, restriction of meiosis to one cell in a cyst[93]		
DAZ-1	Sperm-oocyte switch; Progression through meiosis	Boule	Progression through meiosis	DAZL DAZ BOULE	Licensing & meiotic initiation (DAZL)[99,100] Completion of meiosis, oocyte-to-zygote transition[163]
		Bam	Mitosis to meiosis transition		
		Bgcn			
SmE, SmG	Germline specification	Sm	Germline specification	Sm	Sm proteins associate with TDRD1[164]

*Table refers to proteins mentioned in the text and their homologs, and is not comprehensive. Homologous proteins from different species are listed in the same row.

Abbreviations: GLH (Germ Line Helicase), DDX4 (DEAD (Asp-Glu-Ala-Asp) boX polypeptide 4), MVH (Mouse Vasa Homolog), PGL (P-GranuLe abnormality), OMA (Oocyte MAturation defective), NOS (NanOS related), NANOS2 (Nanos homolog 2), NANOS3 (Nanos homolog 3)

FBF (Fem-3 mRNA Binding Factor), PUM2 (Pumilio 2), GLD (defective in Germ Line Development), FOG (Feminization of Germline), Orb (oo18 RNA-binding protein), DAZ (Deleted in AZoospermia), DAZL (DAZ-like), Bam (Bag of marbles), Bgcn (Benign gonial cell neoplasm, Sm (Small nuclear ribonuclear protein)

Glossary Terms

GAMETES Haploid, differentiated germ cells: mature sperm and eggs.

ZYGOTE One-celled embryo, the initial product of fertilization.

GERM PLASM Specialized cytoplasm that contains factors necessary and sufficient for germ cell specification. May or may not have a known physical correlate in a given species.

P GRANULES Cytoplasmic structures comprising the germ plasm in *C. elegans.*

POLAR GRANULES Cytoplasmic structures comprising the germ plasm in *Drosophila.*

EPIBLAST A cup-shaped sheet of cells derived from the inner cell mass that will eventually form all tissues of the embryo proper.

EXTRAEMBRYONIC ECTODERM Ectodermal tissue that is derived from the epiblast but does not contribute to the embryo proper. Ectoderm is one of the three primary germ layers produced during early embryonic development.

ANTERIOR VISCERAL ENDODERM (AVE) Cell layer underlying the epiblast in the mouse embryo. It does not contribute to the embryo proper but serves important signaling functions during embryogenesis.

PRIMORDIAL GERM CELLS (PGCS) Term used for cells early in the germ cell lineage, before they have initiated meiosis or begun sex-specific differentiation.

GASTRULATION Process by which the three primitive germ layers are formed in the early embryo; one of the first major differentiation events in development.

PREINITIATION COMPLEX Protein complex made up of general transcription factors that positions RNAPII at gene transcription start sites and positions DNA in the RNAPII active site.

POLYCOMB Chromatin regulatory complex that represses gene expression; associated with deposition of H3K27me3.

GERMLINE STEM CELLS (GSCS) Proliferative cells that maintain germ cell production in the adult, often by dividing to produce one self-renewing and one differentiating daughter cell.

NICHE A microenvironment that promotes maintenance of the germline stem cells. The term may refer to the somatic cells responsible for creating this microenvironment, or to the physical location in which they reside.

DISTAL TIP CELL Specialized somatic cell comprising the germ cell niche in *C. elegans.*

SPERMATOCYTE Male germ cell that has initiated meiosis.

OOCYTE Female germ cell that has initiated meiosis. Because meiosis is not complete in the oocyte until fertilization, mature female gametes are oocytes.

OOGENESIS Process of oocyte generation, from mitotic precursor cell to mature oocyte in meiotic arrest.

ECLOSION The transition from pupa to adult in insects: hatching from the pupal case.

CAP CELLS Somatic cells that, together with terminal filament cells and escort cells make up the germ cell niche in *Drosophila* females. They directly contact GSCs and promote stem cell maintenance.

HUB Cone-shaped group of somatic cells comprising the germ cell niche in *Drosophila* males.

CYSTOBLAST GSC daughter cell that has moved away from the niche and initiated differentiation.

LICENSING Process permitting PGCs to respond to signals promoting meiosis and male/female differentiation.

H3.3 Histone H3 variant subunit associated with actively transcribed genes as well as with specific heterochromatic regions such as telomeres. Unlike H3.1 and H3.2, deposition is cell-cycle independent.

AUTOPHAGY Process by which a cell degrades its own organelles or cytoplasmic components, using the lysosomal machinery.

PROTAMINES Highly basic, arginine-rich proteins that replace histones in packaging the genomes of haploid sperm. Packaging with protamines results in highly condensed genomic DNA.

SPERMIOGENESIS The process of differentiation in haploid sperm after meiosis has been completed, involving nuclear compaction, loss of cytoplasm, and generation of a flagellum.

SPERMATOGENESIS Refers to the entire process of sperm generation from mitotic precursor to mature sperm.

PRONUCLEUS Term for the nuclei of the male and female gametes after they have formed a single cell at fertilization, before the nuclei have fused.

RESIDUAL BODY Anucleate cytoplasmic structure remaining after budding of *C. elegans* spermatids.

EPIBLAST-LIKE STEM CELL (EPISC) Stem cells derived from the epiblast of postimplantation embryos, exhibiting a more restricted differentiation potential than naïve embryonic stem cells.

Note: The stages of meiotic prophase (below) are mentioned in the caption for Figure 4; please put them as close to Figure 4 as possible.

PROPHASE First phase of the meiotic or mitotic cell division (M phase), during which chromosomes condense. In meiosis, prophase occurs before meiosis I and is divided into the leptotene, zygotene, pachytene, diplotene, and diakinesis stages.

LEPTOTENE First stage of meiotic prophase. Chromosomes begin to condense.

ZYGOTENE Second stage of meiotic prophase. Homologous chromosomes pair.

PACHYTENE Third stage of meiotic prophase. Homologous chromosomes are tightly held together by the synaptonemal complex, and homologous recombination ('crossing over') begins.

DIPLOTENE Fourth stage of meiotic prophase. The synaptonemal complex breaks down, but homologous chromosomes are held together at sites of recombination.

DIAKINESIS Final stage of meiotic prophase. Chromosomes condense further, the nuclear envelope breaks down, and the meiotic spindle begins to form.

Acknowledgements
The authors would like to acknowledge Dirk de Rooij for useful discussions during preparation of this review, and R. Desgraz, T. Endo, A. Godfrey, J. Hughes, M. Kojima, J. Mueller, and K. Romer for comments on the manuscript.

Competing interests statement
The authors declare that they have no competing financial interests.

Online links

FURTHER INFORMATION
Link 1: http://www.sciencedirect.com/science/article/pii/S0012160601904361
Confocal movies associated with Molyneaux et al 2001, showing migration of living, fluorescently-labeled primordial germ cells in the mouse embryo.

Access to this interactive links box is free online.

Online summary

Genetics of germ cell development

Bluma J. Lesch and David C. Page

Abstract will be imported from review text

Summary

● Germ cells are specialized cells that are responsible for transmitting the genome of an individual organism to its offspring.
● The defining characteristic of the germ cells is their ability to undergo meiosis, in which the diploid genome is reduced to a haploid genome that can combine with another haploid genome at fertilization.
● Many of the factors specifying germ cell identity are RNA binding factors, and many of these RNA binding factors are conserved in the germ cells across multiple species.
● Maintenance of a transcriptionally repressed state is a characteristic of early germ cells in multiple species. Repression is accomplished both by direct inhibition of RNA polymerase II and by establishment of a repressive chromatin configuration.
●The decision to stop mitotic proliferation and enter meiosis is timed differently in the different species and in different sexes of the same species. In some cases, a proliferative pool of germline precursors is retained after this decision, and in some cases all available germ cells enter meiosis together.
● The later steps of germ cell development set up the cues that will guide the earliest stages of embryogenesis.
● Germ cells represent the closest *in vivo* equivalent to *in vitro* pluripotent stem cell systems; understanding germ cell biology will provide new insights into the nature of pluripotency.

about the author

Bluma J. Lesch is a postdoc in David Page's laboratory. After receiving her B.S. from Yale University, she completed her Ph.D. at Rockefeller University, working in Cori Bargmann's laboratory to understand neuronal fate maintenance in *C. elegans*, and her M.D. at Weill Cornell Medical College. She now studies the chromatin-based regulation and the role of the sex chromosomes during major developmental transitions in the mouse germ cells.

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For more information on the authors visit http://pagelab.wi.mit.edu/

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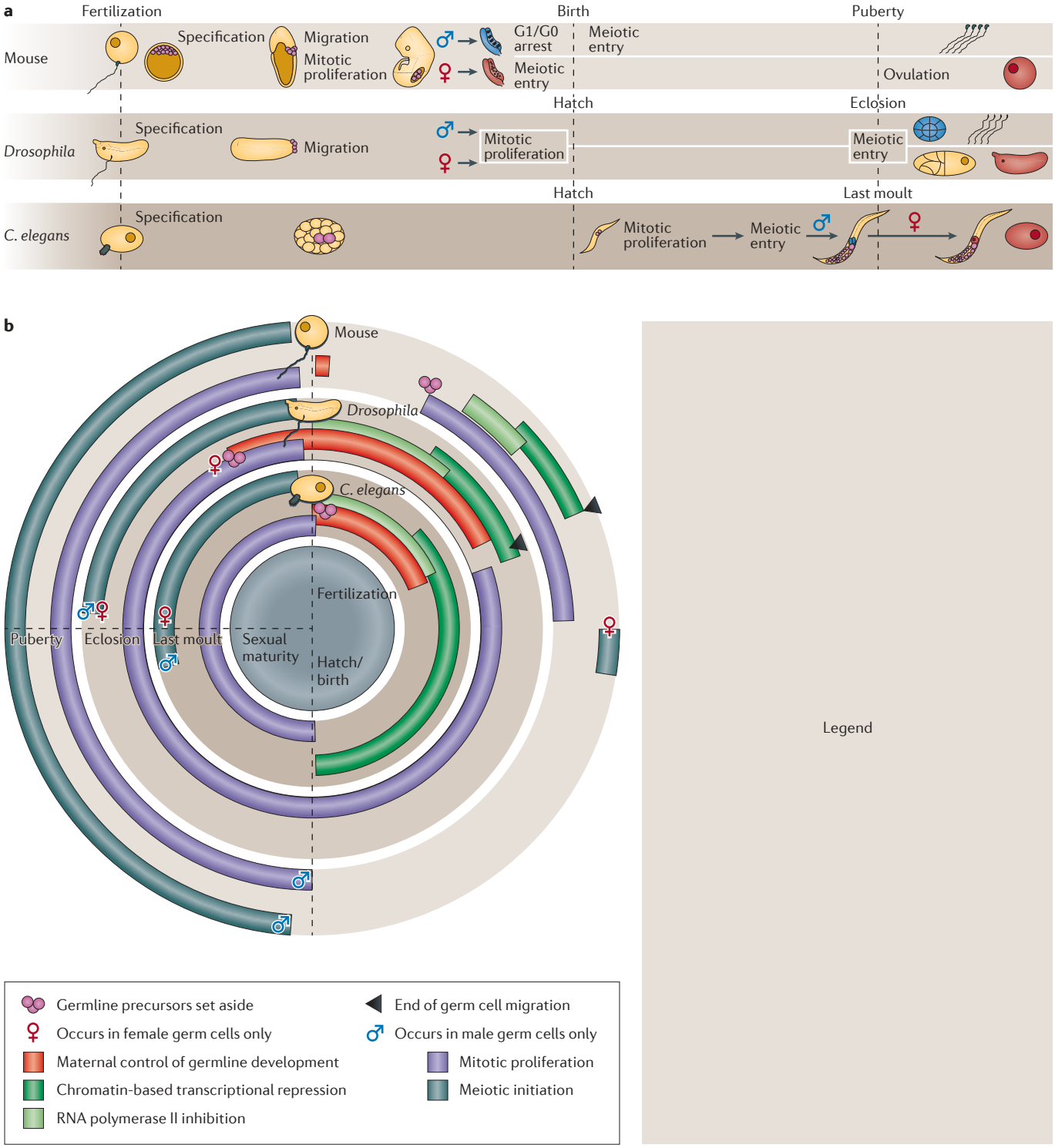

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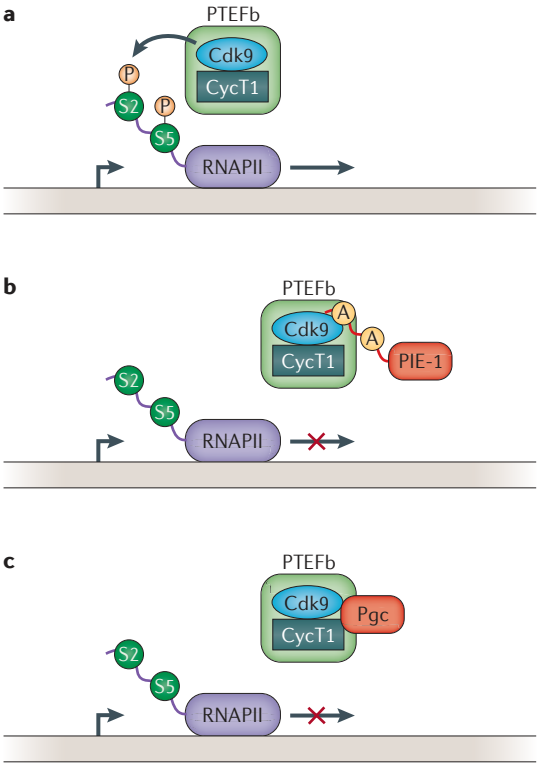

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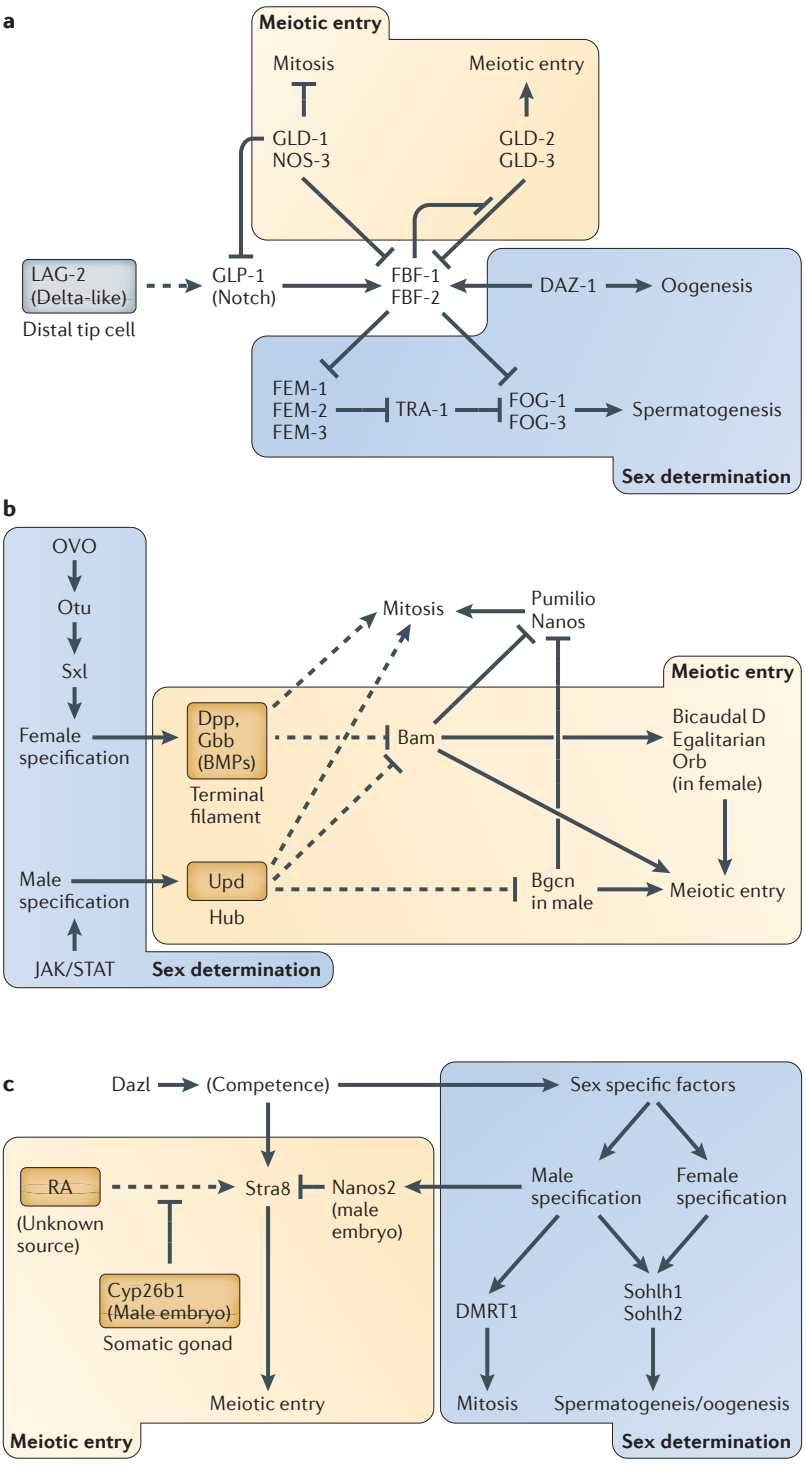

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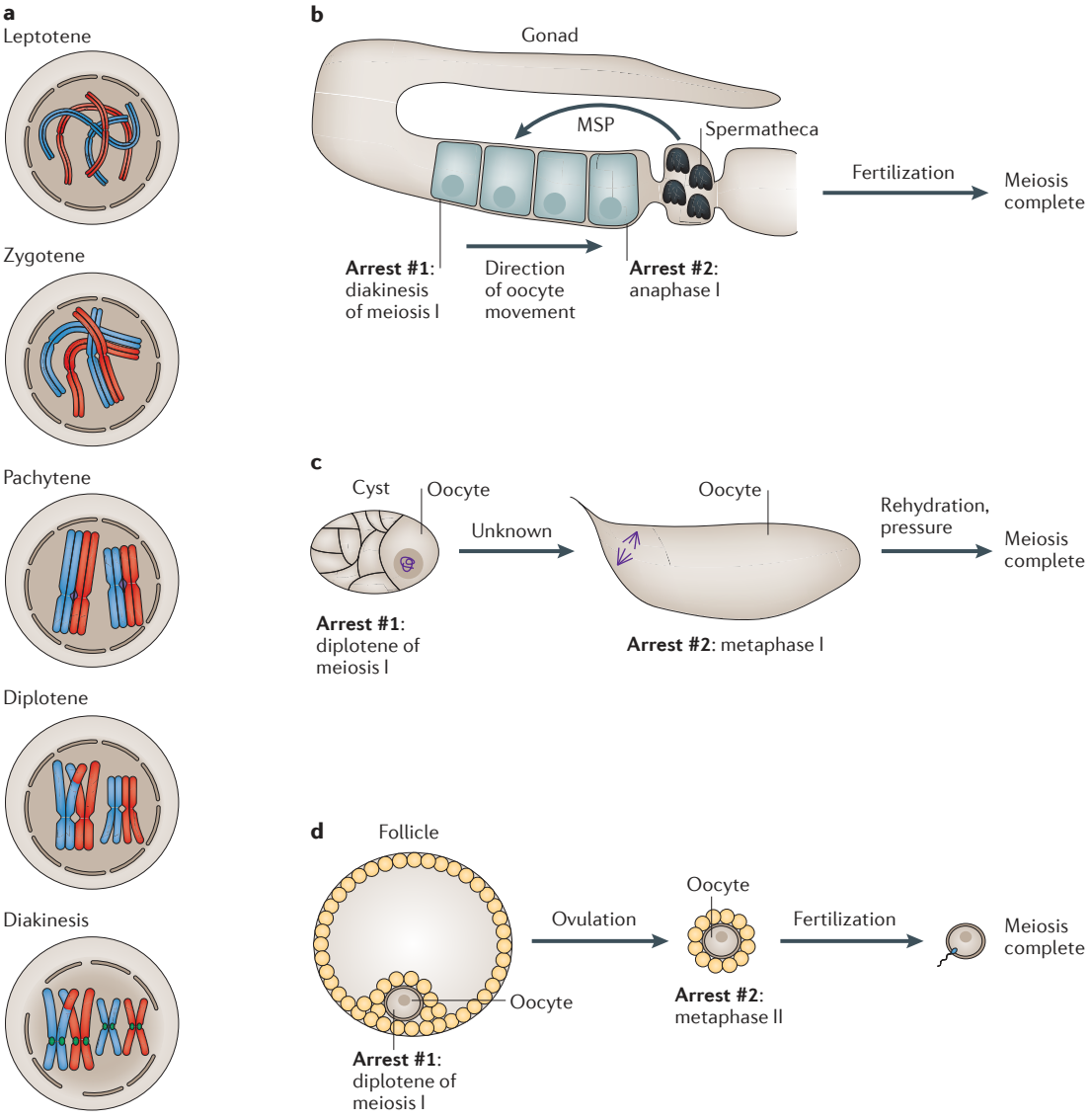

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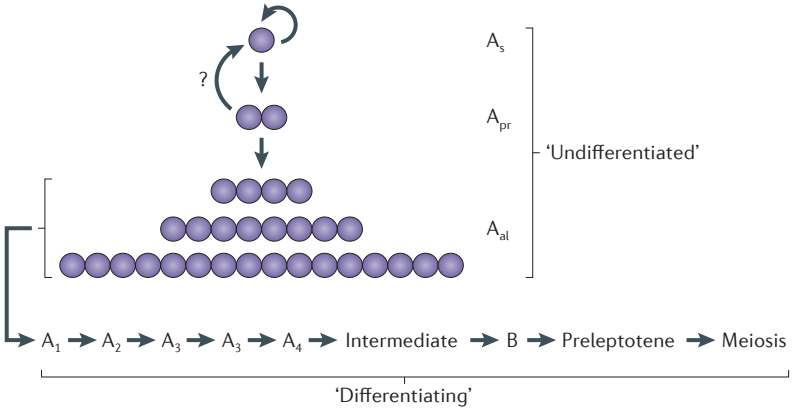

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