Genetics of germ cell development

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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 model organisms *Caenorhabditis elegans*, *Drosophila melanogaster* and the mouse display striking similarities, as well as major differences, in the means by which they control germ cell development. Recent developments in genetic technologies allow a more detailed comparison of the germ cells of these three organisms than has previously been possible, shedding light not only on universal aspects of germline regulation, but also on the control of the pluripotent state *in vivo* and on the earliest steps of embryogenesis. Here, we highlight themes from the comparison of these three alternative strategies for navigating the fundamental cycle of sexual reproduction.

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

Zygotic
Referring to a zygote, which is a one-celled embryo as the initial product of fertilization.

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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. However, to provide this continuity the cells that physically carry the genome from parent to offspring — the germ cells — must negotiate an intricate series of developmental processes in 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 that was previously established in *Caenorhabditis elegans* and *Drosophila melanogaster*. Knowledge that is 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 dialogue 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 summarize the genetic control of germ cell development in *C. elegans*, *D. melanogaster* and the mouse, underlining similarities and differences in the ways these animals arrange for the passage of their genomes from one generation to the next (FIG. 1). We will discuss four tasks that are 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 the regulation of early zygotic processes after fertilization. We will focus on the 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 germ line

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

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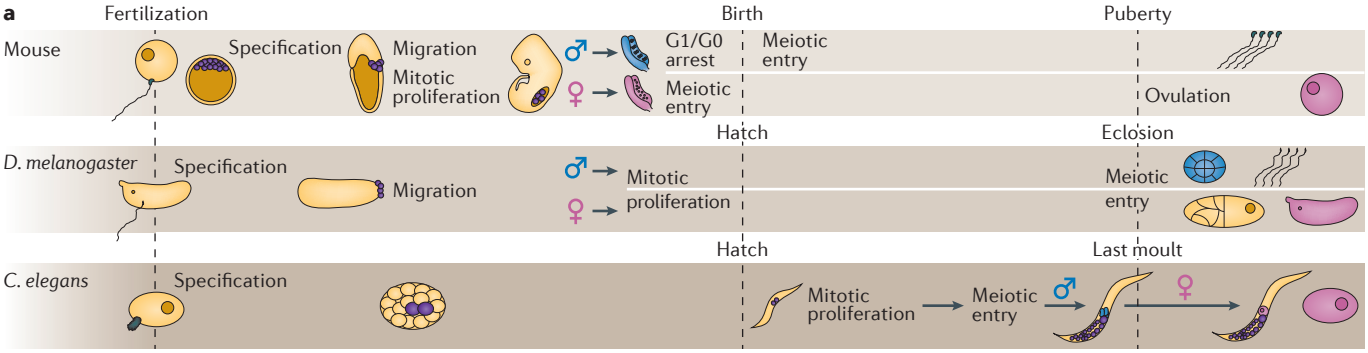

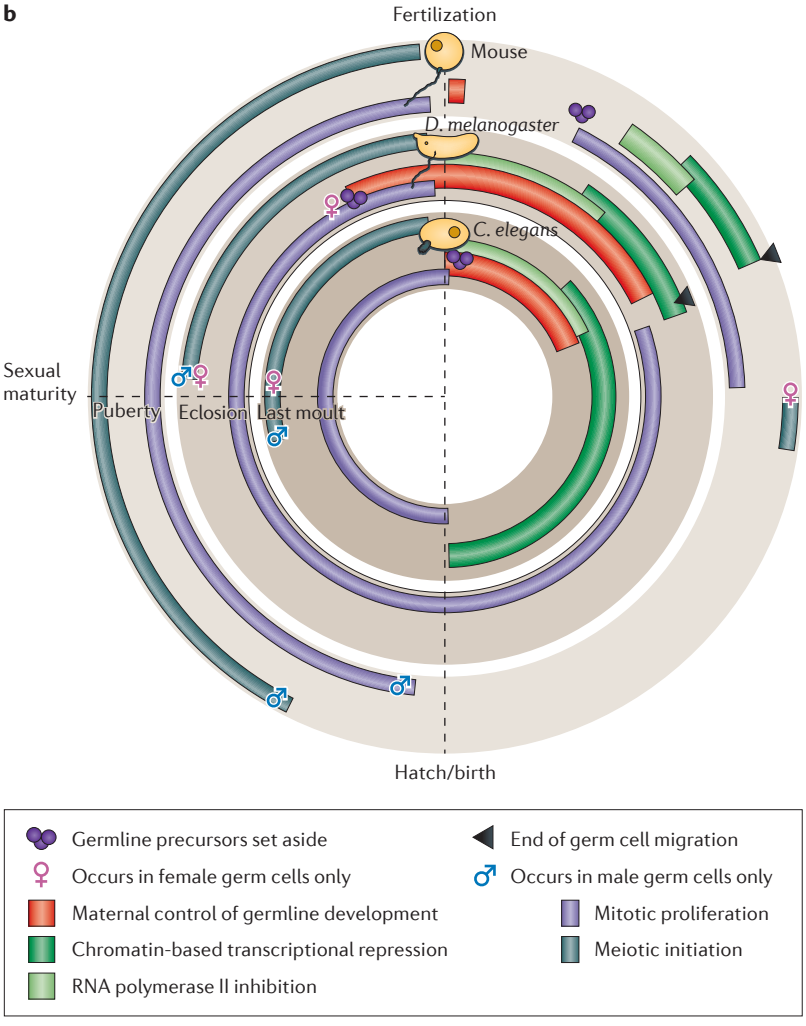

Figure 1 | **Summary of germ cell development in *C. elegans*, *D. melanogaster* and the mouse.** **a** | Germ cell development in *Caenorhabditis elegans*, *Drosophila melanogaster* and the mouse. Timescales are aligned by developmental stage rather than chronological time. Primordial germ cells before the initiation of gametogenesis are shown in purple. Differentiating female gametes are shown in pink and male gametes in blue. In the mouse, the germ line is specified from the epiblast by inductive signals from the surrounding tissue. The primordial germ cells 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 arrest in a G0-like state, 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. Note that in *D. melanogaster*, germline precursors are set aside and maternal control of the germ line begins during oogenesis, overlapping completion of the previous cycle of germline development.

population by signals arising from surrounding tissue[5]. Among the three organisms discussed here, worms and flies exhibit preformation, whereas mice use induction. Importantly, although this division falls along the vertebrate–invertebrate line among these three organisms, this distribution cannot be generalized. As in mice, all mammals generate their germ cells by induction. However, other vertebrates such as zebrafish and *Xenopus laevis* set aside a germ plasm by preformation, and many invertebrates, including annelid worms, molluscs and many insects, segregate their germ line using inductive mechanisms[5].

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

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

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

Caenorhabditis elegans		*Drosophila melanogaster*		Mouse	
Protein	**Role**	**Protein**	**Role**	**Protein**	**Role**
GLH-1, GLH-2, GLH-3, GLH-4	P-granule component	Vasa	Polar granule component	DDX4	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-plasm assembly[11]		
NOS-1, NOS-2, NOS-3	Transcriptional repression[45]; meiotic initiation[159]; sperm–oocyte switch (NOS-3)[61]	Nanos	Transcriptional and translational repression[45,160]	NANOS2, NANOS3	Inhibition of meiosis in male embryos[108]; maintenance of migrating primordial germ cells[107]
FBF-1, FBF-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 spermatogenesis	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 and meiotic initiation (DAZL)[99,100]; completion of meiosis; oocyte-to-zygote transition[163]
		BAM, BGCN	Mitosis-to-meiosis transition		
Spliceosomal proteins (SmE, SmG)	Germline specification	Spliceosomal proteins (SmB, SmD1, SmD2)	Germline specification	Spliceosomal proteins (SmB, SmD)	Sm proteins associate with TDRD1 (REF. 164)

*Table refers to proteins mentioned in the text and their homologues, and is not comprehensive. Homologous proteins from different species are listed in the same row. BAM, Bag of marbles; BGCN, Benign gonial cell neoplasm; DAZ, deleted in azoospermia; DAZL, DAZ-like; DDX4, DEAD-box polypeptide 4, also known as mouse Vasa homologue (MVH); FBF, *fem-3* mRNA-binding factor; FOG, feminization of germ line; GLD, defective in germline development; GLH, germline helicase; NOS, Nanos-related; OMA, oocyte-maturation-defective; ORB, oo18 RNA-binding protein; PGL, P-granule abnormality; piRNA, PIWI-interacting RNA; PUM2, Pumilio 2; Sm, small nuclear ribonuclear proteins (named after the Smith antigen); TDRD1, Tudor domain-containing protein 1.

aside to form the germ line. Germ plasm complexes are composed of RNA and protein; many of the proteins are RNA-binding factors that are involved in translational regulation of germ plasm mRNAs, and many of these RNA-binding proteins are conserved in germ cells across multiple species[6] (TABLE 1).

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, the P_4 cell (the precursor of all germ cells in the adult animal) divides to form the two germline founder cells, Z2 and Z3 (REF. 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 germline helicase 1 (GLH-1), GLH-2, GLH-3 and GLH-4, the P granule assembly proteins PGL-1, PGL-2 and PGL-3, and the meiotic regulators OMA-1 and OMA-2 (REF. 6). Depletion or loss of mRNA or protein components of P granules results in sterility, although frequently two or more redundant family members must be eliminated to achieve complete penetrance. However, mutant embryos that fail to segregate P granules asymmetrically during embryogenesis are still able to segregate specific germ plasm components asymmetrically into the germ cell lineage, and in adulthood these mutant animals are fertile and assemble P granules only in the germ line[8]. Thus, whereas germ plasm components are central to germline development, 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

P granules
Cytoplasmic structures comprising the germ plasm in *Caenorhabditis elegans*.

Oocytes
Female germ cells that have initiated meiosis. Because meiosis is not complete in the oocyte until fertilization, mature female gametes are oocytes.

Polar granules
Cytoplasmic structures comprising the germ plasm in *Drosophila melanogaster*.

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Oskar is necessary and sufficient for the 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, similarly 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 mRNAs such as *nanos* and polar granule component (*pgc*, see below) that are required for pole cell formation, specification and function[10–14]. In addition, the Sm proteins, which are RNA-binding proteins that make up the spliceosome, have a splicing-independent role in specification of the germ line in both *D. melanogaster* and *C. elegans*[15–17].

Induction of the germ line in the mouse. In the mouse, all cells in the embryo are capable of contributing to the germ line until after the blastocyst stage, indicating that there is no specific germ cell lineage that is 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 known as *Blimp1*) and *Prdm14*, are expressed independently of each other in approximately six cells in the proximal-posterior epiblast, close to the extra-embryonic ectoderm, at around embryonic day 6.25 (E6.25)[18,19]. These few cells are recruited from a larger population of cells that are competent, but not destined, to form the germ line, and these cells can be identified by expression of the transmembrane protein IFITM3 (also known as Fragilis)[20,21]. The 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 signalling peptide bone morphogenetic protein 4 (BMP4), which is produced in the extra-embryonic 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 extra-embryonic ectoderm, oppose the inhibitory anterior signal, setting up a gradient of inhibition and supporting the induction of germ cells in the posterior epiblast[20,24–26]. During this time, the expression of the pluripotency-associated genes *Oct4*, *Sox2* and *Nanog* is progressively restricted to the germ line and becomes germ-cell-specific at E8.0 (REFS 27–29).

The means by which a limited subset of cells is initially selected from among the larger number of competent cells that are in the correct position of the posterior epiblast is unclear. Of the genes that are 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 that is similar to that of germ cell precursors — known as primordial germ cells — but these epiblast cells fail to progress past the early stages of specification and eventually die[18]. In *Prdm14* mutants, epiblast cells make it further in development as primordial germ cells, but these cells also eventually die[19]. The early germ cell marker developmental pluripotency-associated 3 (DPPA3; also known as stella) is first detected in the germ cell precursor population at E7.0. DPPA3 expression 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 (miRNA) *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 LIN28 is not sufficient to specify germ cell fate[31]. Likewise, OCT4 is required to form DPPA3-expressing primordial germ cells 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 among worms, flies and mice. 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 the mouse — during a fairly short time 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.

Requirement for transcriptional repression

Following specification, germ cell precursors in all three species are dependent on stringent regulation of gene expression to prevent the 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 that are involved in somatic tissue specification. Germ cells in the mouse and in *D. melanogaster* (but not in *C. elegans*) also spend this period migrating long distances through the embryo, travelling from their site of specification to the region of the developing gonad (reviewed in REF. 1). They must resist an ever-changing set of external cues, while retaining an 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).

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

Extra-embryonic 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 signalling functions during embryogenesis.

Primordial germ cells
A term used for cells early in the germ cell lineage, before they have initiated meiosis or begun sex-specific differentiation.

Gastrulation
The process by which the three primitive germ layers are formed in the early embryo; it is one of the first major differentiation events in development.

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Regulation of RNA polymerase II. Germ cell precursors in *C. elegans* 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 germ line does not develop[35,36]. Ordinarily, the kinase complex, positive transcription elongation factor b (PTEFb), recognizes and binds to the RNAPII carboxy-terminal domain (CTD), phosphorylating serine 2 of the CTD to drive transcriptional elongation (FIG. 2a). PIE-1 has a carboxy-terminal tail sequence similar to that of RNAPII and competes for binding of PTEFb. When PIE-1 is present, PTEFb is thought to be recruited away from RNAPII, thus preventing phosphorylation of Ser2 and inhibiting transcriptional elongation[37,38] (FIG. 2b). In addition, PIE-1 uses a different but still undefined mechanism to prevent phosphorylation of Ser5 of the CTD, thereby inhibiting pre-initiation complex formation and transcriptional initiation[39].

Transcription is repressed in the early *D. melanogaster* germ line by a mechanistically similar but independently derived mechanism. As in *C. elegans*, pole cells are transcriptionally inactive until mid-embryogenesis, and the maternal-effect gene *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 approximately the time that zygotic expression begins in the surrounding soma, ultimately resulting in loss of the germ line[41,42]. Like PIE-1, PGC protein interacts with PTEFb, inhibiting its ability to bind to and phosphorylate the carboxy-terminal tail of RNAPII[43] (FIG. 2c). In mice, levels of phosphorylation on serines 2 and 5 of the RNAPII CTD are also reduced in primordial germ cells, 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 levels of Ser2 and Ser5 phosphorylation seen in murine primordial germ cells also occur through global inhibition of PTEFb remains unknown[44].

Regulation of chromatin. Developing germ cells in all three species also use chromatin-based repression. In *C. elegans* and *D. melanogaster*, repressive chromatin configurations take over from PIE-1-based 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-methylated lysine 4 of histone H3 (H3K4me2 and H3K4me3) and acetylated lysine 8 of histone H4 (H4K8ac), and slightly higher levels of the repressive modification H3K27me3 (REFS 45,46). A complex of maternal-effect proteins, including the Polycomb group orthologues MES-2 and MES-6, is required for deposition of the repressive H3K27me3 mark in germ cells: worms lacking any of these complex components have offspring that fail to develop a germ line[46–48]. Likewise, loss of *C. elegans spr-5* — the homologue of the mammalian *LSD1* (also known as *KDM1*) histone demethylase — results in a progressive failure to demethylate H3K4, impaired transcriptional repression, and cumulative sterility over multiple generations. Furthermore, the absence of *C. elegans nos-1*

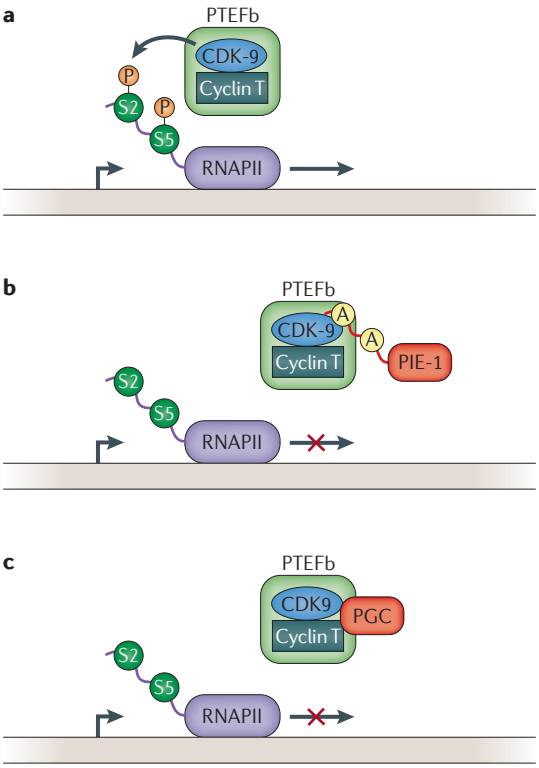

Figure 2 | **Mechanism of transcriptional repression by PIE-1 and PGC. a** | Promotion of transcription initiation and elongation by the positive transcription elongation factor b (PTEFb) complex. PTEFb (light green rectangle) binds to the carboxy-terminal tail of RNA polymerase II (RNAPII), and the cyclin-dependent kinase 9 (CDK-9) subunit phosphorylates serine 2 (green circle) of the tail, allowing transcriptional elongation. **b** | Proposed mechanism for inhibition by PIE-1 in *Caenorhabditis elegans*. PIE-1 (red rectangle) has a carboxy-terminal tail sequence similar to that of RNAPII and competes for PTEFb, thereby inhibiting PTEFb binding to RNAPII and phosphorylation of serines 2 and 5. The PIE-1 carboxy-terminal tail contains alanines (yellow circles) instead of serines at the CDK-9 phosphorylation sites and cannot be phosphorylated, potentially preventing the dissociation of PTEFb from PIE-1 and sequestering PTEFb away from RNAPII. **c** | Inhibition by polar granule component (PGC) in *Drosophila melanogaster*. PGC interacts with both the CDK9 and cyclin T1 components of PTEFb and sequesters the complex away from RNAPII. It is not known whether it acts by a competition mechanism similarly to PIE-1.

Pre-initiation complex
A protein complex made up of general transcription factors that positions RNA polymerase II (RNAPII) at gene transcription start sites and positions DNA in the RNAPII active site.

Polycomb
A chromatin regulatory complex that represses gene expression; it is associated with deposition of the histone mark H3K27me3.

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and *nos-2* — the homologues of *D. melanogaster nanos* — causes premature re-accumulation of H3K4me2 and H3K4me3 in the embryonic germ line and a corresponding germline failure and sterility[45,49]. In *D. melanogaster*, germ cell precursors lacking *nanos* or the *spr-5* and *LSD1* histone demethylase homologue Suppressor of variegation 3–3 (*Su(var)3-3*) also exhibit elevated levels of H3K4me2 and fail to appropriately maintain the 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 primordial germ cell marker, is required for the repression of somatic developmental regulators[51]. PRDM1 associates with the arginine methyltransferase PRMT5, which mediates dimethylation of arginine 3 on histone H2A. This is 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]. However, *Prdm1* mutants maintain expression of actively transcribed germ cell genes, including *Oct4* and *Sox2* (REF. 51). Levels of the repressive H3K27me3 histone modification increase drastically in migrating mouse germ cells at approximately E9.0, following loss of Ser2 and Ser5 phosphorylation on RNAPII, and remain high as RNAPII phosphorylation re-accumulates. Increased deposition of this repressive chromatin mark may serve to maintain a transcriptionally repressed state in the primordial germ cells, 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 that is characteristic of early germ cells, followed by the decision to initiate a sex-specific (into 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, which inhibits meiotic entry and promotes mitotic proliferation (FIG. 3a). LAG-2 signals through GLP-1, a Notch receptor on the GSCs[54,55]. In mutants that are deficient for *glp-1*, GSCs enter meiosis in early L3 and fail to maintain a proliferative germ cell pool; conversely, *glp-1* gain-of-function mutations cause a tumour-like expansion of the germ line[54,56]. Meiotic entry is also regulated by a set of RNA-binding proteins, the Pumilio and FBF (PUF) 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 homologue NOS-3 to coordinate meiotic initiation[57–59]. GLD-3 and NOS-3 inhibit FBF-1 and FBF-2 function, thus 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 and translational regulator, promote spermatogenesis; they are inhibited by the FBF proteins and by TRA-1, a conserved transcription factor that promotes female or hermaphrodite specification in somatic tissues[62–68]. TRA-1 is inhibited by FEM-3, which acts with the additional factors FEM-1 and FEM-2 to push the germ cells towards 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 the 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 the G2 phase of a mitotic cell cycle[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*, sex determination and meiotic entry are temporally separated in *D. melanogaster*. Sex determination occurs in the embryo. Male and female germ cells both 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), which is a major determinant of somatic sexual identity, is transiently expressed in female but not in male primordial germ cells and is cell-autonomously required for female germ cell identity[76] (FIG. 3b). Additional factors, including the ovarian tumour protein (OTU) and the OVO-B isoform of the OVO transcription factor, are also expressed in a female-specific manner in germ cells and act to promote

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

Distal tip cell
A specialized somatic cell comprising the germ cell niche in *Caenorhabditis elegans*.

Spermatocytes
Male germ cells that have initiated meiosis.

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

Oogenesis
The process of oocyte generation, from mitotic precursor cell to mature oocyte in meiotic arrest.

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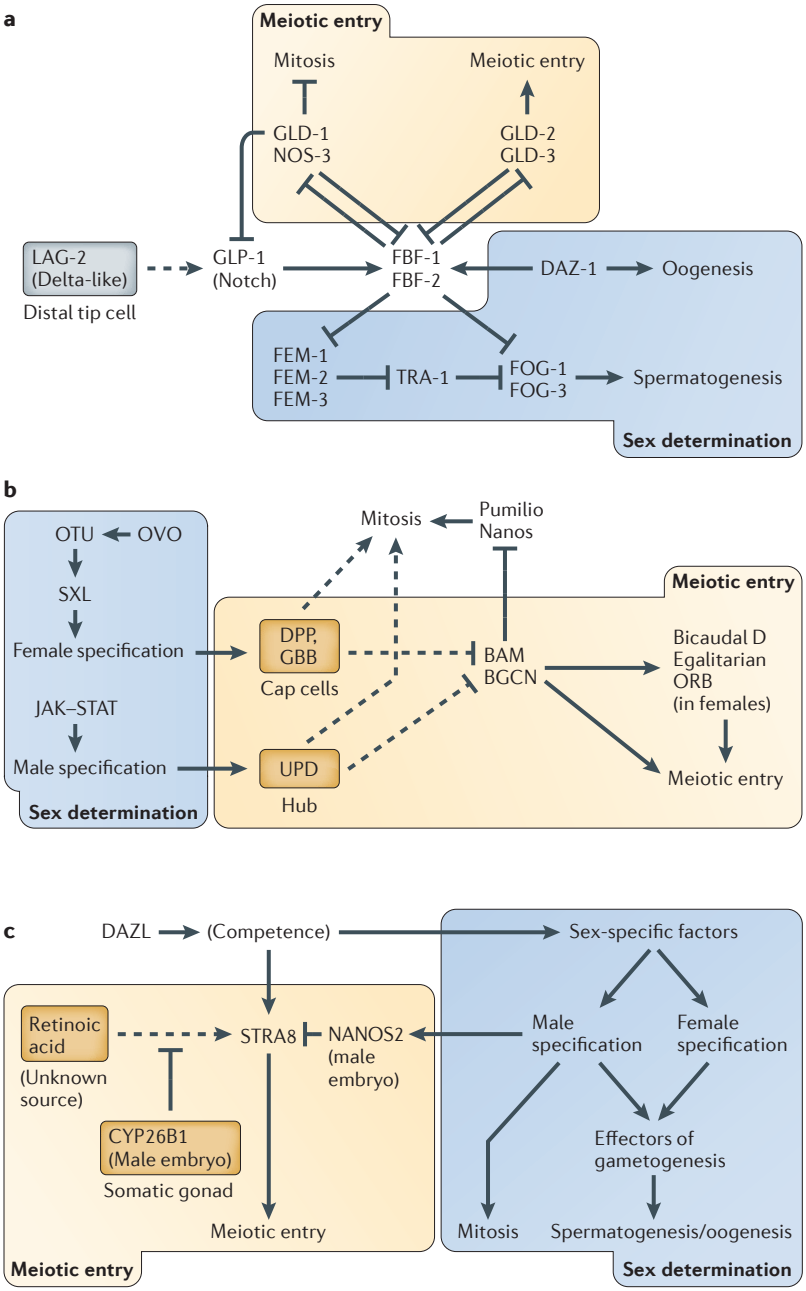

Figure 3 | **Genetic pathways controlling germ cell sex-determination and meiotic entry.** Diagrams show central aspects of each regulatory pathway that are discussed in the main text but do not include all the gene products that have been implicated in these pathways. Gene products in yellow boxes are involved in meiotic entry and those in blue boxes are involved in sex determination. Dotted lines indicate diffusible extracellular signals that weaken when distal to the signal source. **a** | In *Caenorhabditis elegans*, a Notch–Delta signalling pathway promotes mitotic proliferation and germline stem cell (GSC) self-renewal, and entry into meiosis is initiated when GSCs migrate far enough away from the source of the LAG-2 signal. Exit from self-renewal and entry into meiosis at the last (L4) larval stage leads to male (sperm) differentiation, whereas 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 melanogaster*, the sex of the germ cells is determined early in development and specifies the signals that promote self-renewal in GSCs: that is, bone morphogenetic protein (BMP) signals in females (namely, Decapentaplegic (DPP) and Glass bottom boat (GBB)) and signalling through the ligand Unpaired (UPD) in males. The transition to meiosis occurs when the cells leave the somatic niche. In both sexes, Bag-of-marbles (BAM) and Benign gonial cell neoplasm (BGCN) promote cystoblast differentiation and meiotic entry. **c** | In mice, deleted in azoospermia-like (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, whereas the induction of stimulated by retinoic acid gene 8 (*Stra8*) by retinoic acid promotes meiotic initiation. Female germ cells are able to respond to retinoic acid in the embryo, whereas male germ cells are prevented from doing so by CYP26B1 and by NANOS2. In adult males, mitotic self-renewal is promoted[154]. In males and females, various shared effector genes are involved during gametogenesis[155–157]. FBF, *fem-3* mRNA-binding factor; FOG, feminization of germ line; GLD, defective in germline development; JAK, janus kinase; NOS, Nanos-related; ORB, oo18 RNA-binding protein; OTU, ovarian tumour protein; STAT, signal transducer and activator of transcription; SXL, Sex lethal; TRA-1, transformer protein 1.

a female identity[77–79]. Different cell-intrinsic signals, downstream of a JAK–STAT signalling 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: mature sperm are present by eclosion, and mature eggs are present soon afterwards[81,82].

Meiotic initiation and progression are orchestrated by gonad structure. Signalling 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) (FIG. 3b). The RNA-binding proteins Pumilio and Nanos are cell-intrinsically required in GSCs to maintain proliferation and to 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 gonial 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 tumour-like overgrowth of mitotic GSCs[88–90] (FIG. 3b). In both sexes, cystoblasts divide mitotically four times with incomplete cytokinesis to generate 16 linked daughter cells, which are collectively called a cyst[91,92]. In males, all cyst cells enter meiosis and mature as sperm,

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

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whereas in females only some cells in 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 the formation of cysts with no oocyte[93].

Meiotic initiation and sex determination in the mouse. Murine primordial germ cells 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 primordial germ cells begin to reach the developing gonad around E10.5 (REF. 94). Over the next 48 hours, primordial germ cells of both sexes rapidly lose DNA cytosine methylation and initiate the expression of a few shared transitional genes[95–98]. During this transitional period, primordial germ cells undergo licensing, which means that they exit their pluripotent, migratory state and acquire competence to initiate sexual differentiation and enter meiosis (FIG. 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 (FIG. 4a) and begin to differentiate as oocytes, whereas male germ cells arrest in a G0-like state until after birth[101]. As in *C. elegans*, the signals that trigger meiotic entry in males and females are similar but temporally displaced (FIG. 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 helix–loop–helix (HLH) protein stimulated by retinoic acid gene 8 (STRA8)[102,103]. In females, *Stra8* is expressed in germ cells in response to retinoic acid, beginning at E12.5–E13.5, and meiotic prophase begins soon afterwards[104]. In prenatal males, the cytochrome P450 enzyme CYP26B1 degrades retinoic acid, thus preventing the induction of *Stra8* (REFS 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, such that 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. However, although the initiation of meiosis at E13.5 is considered to be equivalent to female specification, transcriptional profiling of male and female germ cells at E12.5 points to a moderate amount of sex-specific transcription one day before 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 that are 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. By contrast, developing oocytes carefully regulate meiotic progression, pausing at specific times to coordinate the completion of meiosis with fertilization. They also maintain high transcriptional activity and expand their cytoplasmic volume 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 major sperm protein (MSP)[111,112]. Although spermatid nuclei condense as in other species, high levels of the histone variant H3.3 and lower levels of the canonical histone H3.1 are retained in mature sperm, as are patterns of H3K4 methylation that were 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 time they grow in size and accumulate maternal RNAs and proteins that are required for early embryogenesis (FIG. 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 re-enter 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 the 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. Completion of meiosis in sperm and aspects of spermiogenesis require *boule*, which is a homologue of *C. elegans daz-1* and mouse *Dazl*[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 that are synthesized

Cap cells
Somatic cells that, together with terminal filament cells and escort cells, make up the germ cell niche in *Drosophila melanogaster* females. They directly contact germline stem cells and promote stem-cell maintenance.

Hub
The cone-shaped group of somatic cells comprising the germ cell niche in *Drosophila melanogaster* males.

Cystoblast
A germline stem cell daughter cell that has moved away from the niche and initiated differentiation.

Licensing
A process permitting primordial germ cells to respond to signals promoting meiosis and male or female differentiation.

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.

Residual body
An anucleate cytoplasmic structure remaining after budding of *Caenorhabditis elegans* spermatids.

H3.3
A histone H3 variant subunit that is 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.

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.

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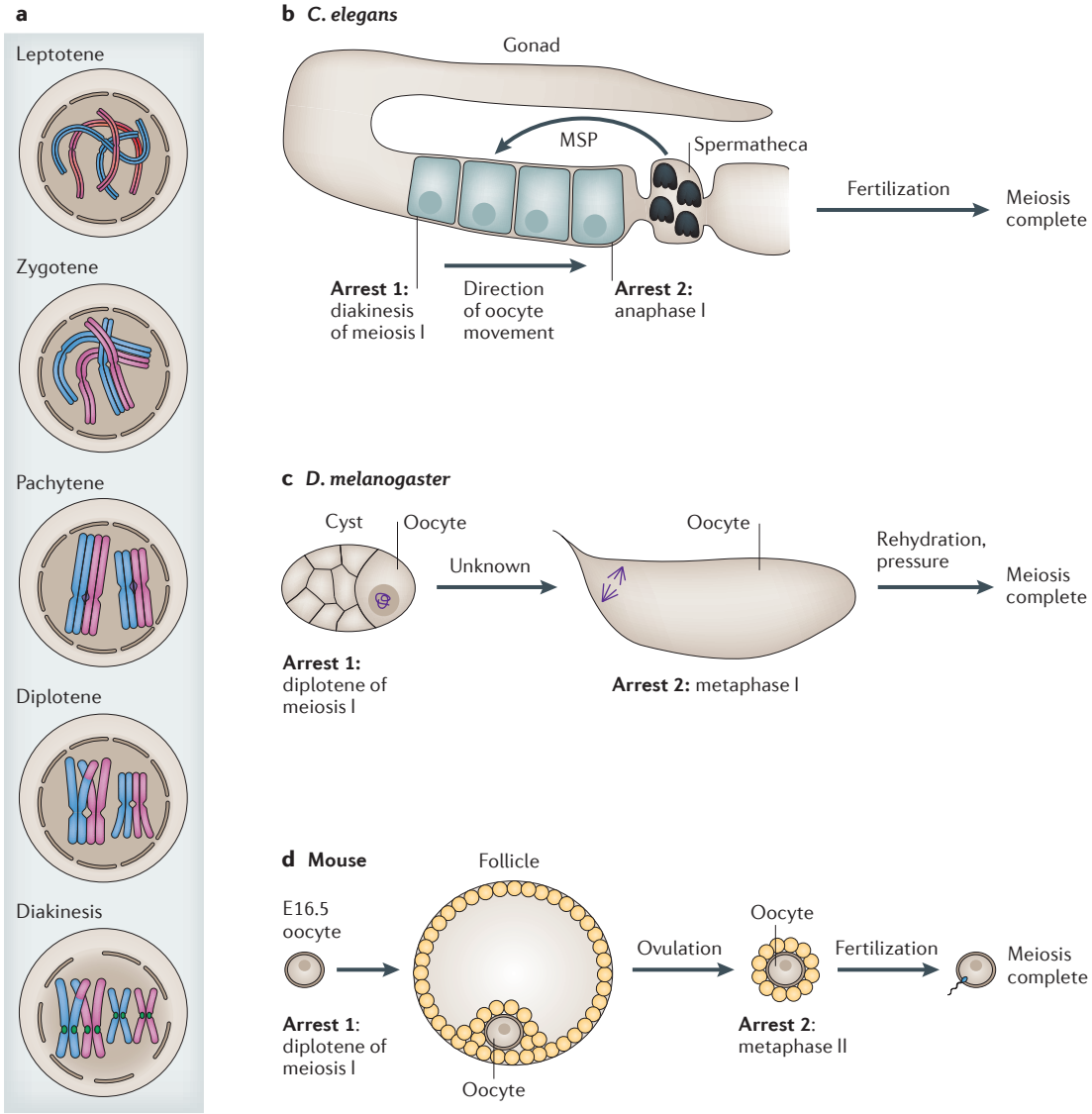
by nurse cells[2] (FIG. 4c). The oocyte is polarized along both the dorsal-ventral and anterior-posterior axes by signalling from overlying follicle cells through the epidermal growth factor receptor (EGFR) ligand Gurken. This polarization positions *bicoid* and *oskar* mRNA gradients for translation following fertilization,



Leptotene
The first stage of meiotic prophase. Chromosomes begin to condense.

Zygotene
The second stage of meiotic prophase. Homologous chromosomes pair.

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

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

Diakinesis
The final stage of meiotic prophase. Chromosomes condense further, the nuclear envelope breaks down and the meiotic spindle begins to form.

Figure 4 | **Meiotic arrest in 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 *Caenorhabditis 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 afterwards, are fertilized and complete meiotic division. **c** | Meiotic arrest in *Drosophila melanogaster*. The cyst consists of the 16 cells that are produced from four mitotic divisions of the founding precursor cell. One of these cells becomes the oocyte, while the remaining 15 cells 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, this arrest is released by an unknown signal and the oocyte then arrests again at metaphase I. This second arrest is relieved by rehydration and mechanical pressure during ovulation. **d** | Meiotic arrest in the 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 re-enter the meiotic cycle. During the first meiotic arrest, the oocyte is surrounded by supporting cells and is 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.

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

which will establish the embryonic anterior-posterior axis and permit rapid progression through the early stages of embryogenesis[121]. Towards the end of oogenesis, unknown signals induce progression from arrest at diplotene of prophase I to metaphase I, when a second arrest occurs. After the oocyte passes through the oviduct, rehydration and pressure trigger passage through the last stages of meiotic division; in contrast to *C. elegans* and the mouse, fertilization is not required for completion of meiosis in the *D. melanogaster* oocyte[2]. After fertilization, the paternal pronucleus rapidly decompacts 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 repackaging of the male pronucleus is crucial to early embryogenesis[122].

Preparation for embryogenesis in the 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 arrest in a G0-like state. Oocytes arrest in diplotene of meiosis I during embryogenesis and remain transcriptionally active, generating a store of proteins and mRNAs to guide the early development of the zygote (FIG. 4d). In sexually mature mice, hormonally triggered ovulation allows the oocyte to proceed from prophase I to metaphase II, with a concurrent pausing of transcription and reduction of translation[110]. At fertilization, the egg completes meiosis, and the female pronucleus remains in a repressed state. By 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].

Perspectives

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.

The regulation of gene expression at both the transcriptional and translational levels is central to germ cell development, and may help to maintain a flexible cell state (TABLE 1). Post-transcriptional control in the germ line operates through several mechanisms, including (but not limited to) the recruitment of mRNAs to the ribosome, the modulation of poly(A) tail length and transcript stability, the restriction of translation to specific regions within the cell, and the 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 have 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 germ line 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 populations of cells. 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 PIWI-interacting RNAs (piRNAs), probably have an additional role in maintaining the balance between repression and flexible gene expression in the germ line (reviewed in REF. 129). Similarly, numerous histone variants and chromatin regulators are specific to the germ line, but their functions are largely unknown[130]. Molecular characterization of the epigenetic state of the germ line will be necessary to understand recent reports invoking 'epigenetic' inheritance (BOX 1).

Box 1 | Transgenerational epigenetic inheritance

Several recent, high-profile studies have called attention to transgenerational epigenetic inheritance in *Caenorhabditis elegans*, *Drosophila 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 offspring and sometimes later generations.

In worms, mutations in the histone demethylase gene *spr-5* (a homologue of mammalian *LSD1*) cause a weak sterility phenotype that is passed on and becomes progressively more severe in subsequent generations, despite no further changes in the *spr-5* coding and promoter DNA sequence[49]. Meanwhile, mutations in three genes encoding a histone methyltransferase 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 both the activity of the transcription factor ATF2 and the 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 that have been fed a low-protein diet exhibit altered liver gene expression profiles, and F1 female offspring of male rats that have been 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 epigenetic regulation and genome organization throughout germ cell development will provide insights into such a mechanism. For example, the white-spotting phenotype of Kit^{tm1Alf}-mutant mice was found to be transmitted by abnormally retained mRNAs in sperm, thus providing a mechanistic basis for this case of non-Mendelian inheritance[146]. However, our current understanding of the regulation of the germ cell epigenome is insufficient to provide true insight into the means by which the effects that have been 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 germ line of each of these model organisms is required to fully understand the meaning of these effects.

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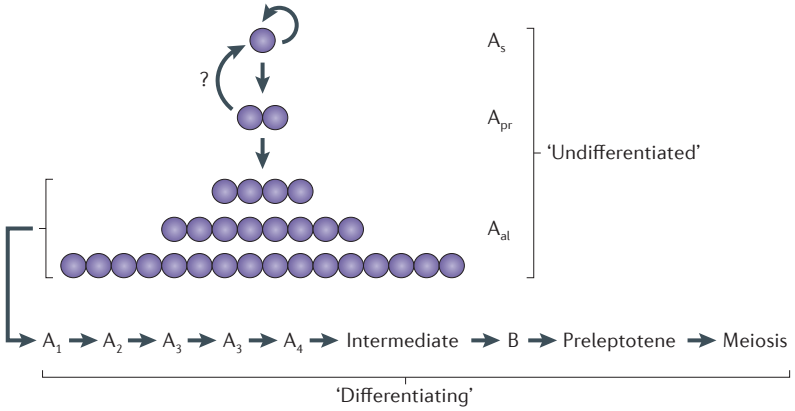
Box 2 | **Spermatogonial stem cells in mammals**



In male mammals, germ cells enter a G0-like arrested state 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 labelled testicular cells are transferred to a recipient testis that has been depleted of germ cells, and are able to re-establish 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 towards 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 precursors to haploid spermatids has been well mapped (see the figure 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_{pr}). These pairs then divide synchronously between one and three times to become linearly conjoined chains of 4–16 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 past 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 cells expressing markers that are associated with differentiating spermatogonia and some expressing markers that are thought to be associated with self-renewal[149]. Long-term lineage-tracing experiments indicate that there are more A_s cells than are required to maintain a testis-wide pool of precursor cells[150]. By contrast, there are not enough A_s cells to provide the numbers of A_{pr} cells that are found in the seminiferous tubule at any given time point[151]. In partial explanation, it now seems 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 (as shown by the question mark in the figure)[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.

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 (FIG. 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 germ lines 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 signalling from a somatic niche[81,131–133]. Owing to 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 that are 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, including OCT4 and SOX2, that are not expressed in any other tissues; and early mammalian embryonic germ cells are capable of generating pluripotent stem cells (known as embryonic germ (EG) cells) in culture[28,29,134]. Primordial germ cells can also give rise to tumours, called teratomas, that produce tissues from all three germ layers, which is 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 of subpopulations within adult germ cell pools promise to provide insights into the balance between pluripotency and differentiation in these cells (BOX 2).

The possibility of generating functional germ cells *in vitro*, especially from patient-derived induced pluripotent stem cells (iPSCs), 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 embryonic stem cells (ESCs) and iPSCs via an intermediate state, called epiblast-like stem cells (EpiSCs), 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.

Epiblast-like stem cells (EpiSCs). Stem cells that are derived from the epiblast of postimplantation embryos; they exhibit a more restricted differentiation potential than naive embryonic stem cells.

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Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
David C. Page's homepage: http://pagelab.wi.mit.edu
Confocal microscopy movies associated with reference 33, showing the migration of living, fluorescently labelled primordial germ cells in the mouse embryo: http://www.sciencedirect.com/science/article/pii/S0012160601904361

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