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Gene expression programs in mammalian spermatogenesis

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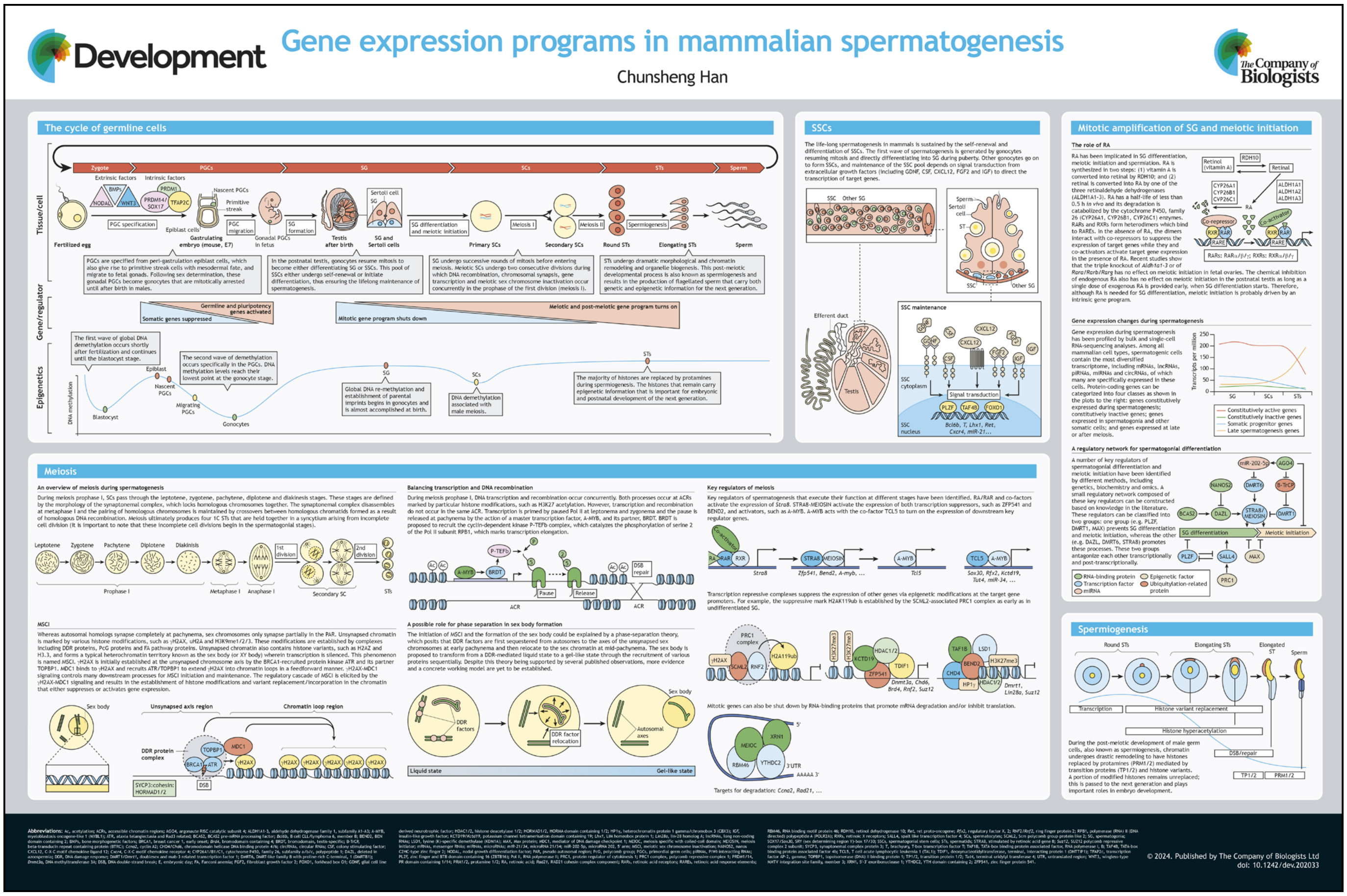
ABSTRACT

Mammalian spermatogenesis, probably the most complex of all cellular developmental processes, is an ideal model both for studying the specific mechanism of gametogenesis and for understanding the basic rules governing all developmental processes, as it entails both cell type-specific and housekeeping molecular processes. Spermatogenesis can be viewed as a mission with many tasks to accomplish, and its success is genetically programmed and ensured by the collaboration of a large number of genes. Here, I present an overview of mammalian spermatogenesis and the mechanisms underlying each step in the process, covering the cellular and molecular activities that occur at each developmental stage and emphasizing their gene regulation in light of recent studies.

KEY WORDS: Spermatogenesis, Meiosis, Spermiogenesis, Primordial germ cell, Gonocyte, Spermatogonium, Spermatogonial stem cell, Spermatocyte, Spermatid

Introduction

Spermatogenesis is the process by which sperm are generated from spermatogonial stem cells via multiple steps, including the mitotic amplification of spermatogonia, the meiosis of spermatocytes and the post-meiotic development of spermatids. It is probably the most complex cellular developmental process, during which molecular events, such as mitosis, meiosis and epigenetic dynamic changes, occur in a highly orchestrated manner. Spermatogenesis involves many cell types and a large number of genes with different expression patterns, and is regulated by a combination of extracellular signals, cell–cell interactions and intrinsic molecular circuits. Research in



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spermatogenesis has been accelerated by the application of diverse technologies, such as mouse genetics, *in vitro* culture of spermatogonial stem cells and various omic methods. However, a complete and coherent molecular model of spermatogenesis has yet to be determined, despite the elucidation of the physiological and molecular functions of more and more genes and the fact that small-scale gene interaction networks are emerging. Here, I present an overview of our current understanding of how the different stages of mammalian spermatogenesis are regulated by programs controlling gene expression. For a better understanding of the overall process, I begin by briefly reviewing the specification and development of primordial germ cells (PGCs). For each stage of spermatogenesis, I describe the formation and development of major cell types, the extracellular signals, the gene expression patterns, the key regulators and their regulatory relationships, and the epigenetic status and changes in spermatogenic cells. I also discuss how the dynamic changes to the epigenetic landscape during spermatogenesis have profound effects, not only on spermatogenic cells, but also on subsequent generations. I do not cover many important aspects of spermatogenesis that are not closely related to gene expression programs, such as endocrine regulation of spermatogenesis, meiotic DNA recombination or the formation of cell type-specific organelles, such as acrosomes and flagella, but these topics have been covered in other reviews (Oduwole et al., 2021; Bhattacharya et al., 2023; Talibova et al., 2022; Pleuger et al., 2020).

Primordial germ cell specification and development

Our knowledge of mammalian PGC specification and development mainly derives from studies in mice (Saitou, 2021). The combined use of cutting-edge methods, such as the *in vitro* culture of epiblasts, lineage-tracing techniques, various omic methods and the *in vitro* induction of germ cells from pluripotent stem cells, has provided a coherent mechanistic framework for PGC specification. Briefly, pre-gastrulation epiblast cells, potentiated by WNT signals, production of which in turn depends on NODAL signaling, respond to bone morphogenetic proteins (BMPs) in a dose-dependent manner. Transcription factors, such as PRDM1, PRDM14 and TFAP2C, are upregulated either directly or indirectly by BMPs and WNT3 signaling and/or by each other and form a core network to upregulate the expression of germ cell genes and pluripotency genes [such as *Nanog*, *Oct4* (*Pou5f1*) and *Sox2*], repress mesoderm genes (such as *T*, which mediates WNT and BMP signaling) and initiate epigenetic reprogramming (Saitou, 2021; Tang et al., 2016). Although the general structure of this three-layer framework, consisting of the intercellular signals, the regulatory network of key transcription factors and the downstream effector genes, seems to hold in PGC specification across humans and perhaps other mammals (Kobayashi et al., 2017, 2021; Kobayashi and Surani, 2018; Sasaki et al., 2016), specific differences have been revealed. For example, SOX17 is crucial for PGC specification in humans but not in mice (Hara et al., 2009), whereas SOX2 is expressed in mouse PGCs but its expression in human PGCs is suppressed by PRDM1 to favor germline specification rather than the neural fate (Kurimoto et al., 2008; Lin et al., 2014). Nascent PGCs start to migrate to the forming fetal gonads soon after their specification. Following sex determination, gonadal PGCs become gonocytes (also known as pro- or pre-spermatogonia) arrested at G_0 in males (Manku and Culty, 2015).

Epigenetic modifications of the genome, such as DNA methylation and chromatin modifications, are reprogrammed on a genome-wide level in mammalian germ cells (Lee et al., 2014). The germline undergoes two major waves of DNA demethylation. The first occurs after fertilization, when the highly methylated sperm genome is actively demethylated before and during the first S phase (Wang et al., 2014). Subsequently, both the paternal and maternal genomes are demethylated passively until the blastocyst stage (Wang et al., 2014). The second wave of demethylation begins when PGCs arise from epiblast cells and continues until they colonize the fetal gonads to become gonocytes (Wang et al., 2014). During migration, PGCs also reduce H3K9me2 and increase H3K27me3 levels (Seki et al., 2005). Methylation starts to be re-established shortly after sex determination in males. DNA methylation is essential for germ cell development, as indicated by the failed meiosis of spermatocytes in mice lacking the DNA methyltransferase genes *Dnmt3a*, *Dnmt3c* or *Dnmt3l* (Bourc'his and Bestor, 2004; Kaneda et al., 2004; Barau et al., 2016). This re-establishment of DNA methylation suppresses retrotransposon activity and establishes genetic imprinting (Bourc'his and Bestor, 2004; Kaneda et al., 2004; Barau et al., 2016). PIWI-interacting RNAs (piRNAs), a class of small RNAs that are specifically expressed in germ cells, also contribute to DNA methylation of retrotransposons, as they act together with their binding proteins to suppress retrotransposon activity by establishing DNA methylation and/or by direct degradation of their complementary RNA targets (Zhou et al., 2022). Orderly and extensive epigenetic reprogramming in PGCs is required for the erasure of epigenetic memories, the resetting of parental imprints, the activation of the gametogenetic gene program, and the reacquisition of underlying totipotency that occurs after fertilization (Tang et al., 2016, 2022).

The establishment and maintenance of the spermatogonial stem cell pool

After birth, during pubertal development, gonocytes resume mitosis and differentiate into spermatogonia (SG) (Manku and Culty, 2015). Although some gonocytes directly become differentiated SG to initiate the first wave of spermatogenesis, others turn into spermatogonial stem cells (SSCs) that undergo either self-renewal or differentiation to ensure the life-long process of spermatogenesis (Yoshida et al., 2006). One prominent feature of spermatogenesis is that cytokinesis in most SSC divisions is incomplete, which generates a syncytium of cells interconnected by cytoplasmic bridges (de Rooij and Russell, 2000). Attempts to identify molecular markers for SSCs were initially unsuccessful because the candidate markers are expressed inconsistently in different subpopulations of SG (de Rooij, 2017). The identity of SSCs therefore remained elusive until a recent study showed that the transcription factor FOXC2 is a specific marker for SSCs (Wang et al., 2023). This study showed that FOXC2 is only expressed in a small fraction of undifferentiated SG in mice. These $FOXC2^+$ cells undergo both symmetric and asymmetric divisions, and can fragment off the SG syncytium. $FOXC2^+$ cells are usually quiescent and are essential for maintaining the SSC pool. They contribute to almost all spermatogenesis. This work has not only identified a specific marker for SSCs, but also demonstrated that SSCs in mammals undergo asymmetric divisions, as in flies, which explains the balance between self-renewal and differentiation in the simplest way (Wang et al., 2023).

The maintenance of the SSC pool depends on both extracellular and intrinsic factors. These factors have been identified through mouse genetics, cultured SSCs and transcriptomic data (Kubota and Brinster, 2018). Extracellular growth factors, including GDNF, FGF, CSF, IGF and CXCL12 are secreted by testicular somatic cells (such as Sertoli cells inside seminiferous tubules, peritubular myoid cells, interstitial cells and cells from circulation systems) and/or the SSCs themselves to promote SSC proliferation (Kubota and Brinster, 2018). For example, inadequate amounts of GDNF in mice leads to the depletion of SSCs whereas GDNF overproduction results in the formation of germ cell tumors, and GDNF is essential for the long-term culture of SSCs in all examined mammals (Meng et al., 2000). Transmembrane receptors and their downstream signal transducers are activated by GDNF, and many of their target genes have been found to be important for SSC self-renewal (Kubota and Brinster, 2018). However, some transcription factors, including PLZF (ZBTB16), TAF4B and FOXO1, are essential for SSC maintenance and probably execute their function independently of GDNF signaling (Song and Wilkinson, 2014). Transplantation of SSCs from species such as rat, dog, cow, pig and baboon into the testes of immunodeficient mice has suggested that the composition and function of the SSC niche is conserved across these species (Brinster, 2002).

Spermatogonial differentiation and meiotic initiation

SG undergo successive rounds of mitosis and ultimately reach a point of no return to be committed to meiosis (Sou et al., 2021). The commonly accepted model for meiotic initiation is that retinoic acid (RA) induces meiosis, and substances such as CYP26B1, which degrades RA, inhibit meiosis (Bowles and Koopman, 2007; Spiller and Bowles, 2019). However, this model has recently been challenged, as fetal germ cells lacking all three RA-synthesizing enzymes or RA receptors still initiate meiosis (Chassot et al., 2020; Vernet et al., 2020). Moreover, a recent study in the mouse testis that used a chemical inhibitor of RA synthesis to block *in vivo* RA signaling before administration of a single injection of exogenous RA showed that RA is required only for SG differentiation but not for meiotic initiation (Kirsanov et al., 2023). It will therefore be important to elucidate how the intrinsic molecular program, triggered in response to RA at the beginning of SG differentiation, promotes meiotic initiation.

Based on gene expression patterns in mouse spermatogenesis from a number of transcriptomic studies (Soumillon et al., 2013; Gan et al., 2013; Lin et al., 2016; Hasegawa et al., 2015), genes can be divided into four classes: genes that are somatic developmental regulators and that are not expressed during spermatogenesis (constitutively inactive genes); genes that are expressed in SG and somatic cells but are silenced in late spermatogenesis (somatic/progenitor genes); genes that are highly expressed in spermatocytes and spermatids (late spermatogenesis genes); and genes that are constitutively active during spermatogenesis (constitutively active genes) (Hasegawa et al., 2015; Maezawa et al., 2018a). Many late spermatogenesis genes already start to express in SG in response to RA signaling (Kirsanov et al., 2023). Our previous work showed that, 5 days after being treated with a single dose of RA for 24 h, mouse SSCs cultured on feeder cell-free plates differentiate to generate spermatocytes in the leptotene/zygotene stage of prophase of meiosis I, and as many as 4600 genes show altered expression in response to RA (Wang et al., 2016). Gene expression patterns, the associated cell types and their evolutionary features across different species have been revealed by single-cell RNA-sequencing studies in which cell states and pseudo time courses of the transitions of these states can be computed guided by knowledge in the expression of known marker genes (see Rabbani et al., 2022 for a nice review on this topic). The degree of conservation of gene programs across species is the highest in SG, drops when cells enter into meiosis, and reaches the lowest levels of conservation towards the end of spermatogenesis (Shami et al., 2020; Murat et al., 2023).

A limited number of key regulators of SG differentiation and meiotic initiation have been identified in the past two decades and their regulatory relationship has been reviewed elsewhere (Chen et al., 2021). These regulators can be divided into two functional groups: those maintaining the stem cell pool (PLZF, MAX, DMRT1, NANOS2, AGO4, miR-202-5p) and those promoting SG differentiation [BCAS2, DAZL, STRA8, MEIOSIN, DMRT6 (DMRTB1), SALL4, b-TrCP (BTRC)]. Regulators within the same group tend to cooperate to promote each other's expression and/or activity, whereas regulators between groups antagonize each other's expression and/or function (Chen et al., 2021). As SG undergo differentiation, heterochromatinization increases and chromatin accessibility decreases (Vara et al., 2019). Chromatin interaction results in topologically associating domains (TADs), and chromatin reorganization during spermatogenesis is reflected by the dissolution and restoration of these structures during and after meiosis, respectively (Wang et al., 2019). During the mitosis–meiosis transition, accessible chromatin in spermatogonia is largely closed and *de novo* formation of accessible chromatin takes place in meiotic prophase, with these changes occurring mostly in intergenic and intronic regions containing regulatory elements (Maezawa et al., 2018b). For somatic/progenitor genes on autosomes, the repressive marker H3K27me3 (but not H3K9me2) is gradually established on their transcription start sites (TSSs) during the pachytene spermatocyte to round spermatid transition (Sin et al., 2015). Meanwhile, the activating marker H3K4me2/3 remains on their TSSs (Sin et al., 2015). This H3K27me3-H3K4me2/3 bivalent marker modification is believed to ensure gene silencing during late spermatogenesis and a quick re-activation in embryos after fertilization (Sin et al., 2015). For late spermatogenesis genes, RNA polymerase II (RANPII), H3K4me2 and H3K4me3 are already present on their TSSs in undifferentiated SG; the H3K27me3 level on these TSSs is also lower than that on the constitutively inactive genes and higher than that on the somatic/progenitor genes and the constitutively active genes at this stage, indicating that the late spermatogenesis genes are poised for activation as early as in undifferentiated SG and that their temporal activation during spermatogenesis could be ensured by additional activating markers, such as histone lysine crotonylation and H4K8ac (Sin et al., 2015).

Meiosis of spermatocytes

During prophase of meiosis I, homologous chromosomes undergo condensation, synapsis/desynapsis, recombination, and crossover formation. This is followed by segregation into two secondary spermatocytes, each of which quickly completes a second division analogous to mitosis to generate haploid spermatids. The meiosis I prophase is a prolonged G2 phase of the cell cycle, during which cells pass through the leptonema, zygonema, pachynema, diplonema and diakinesis subphases. The basic events occurring in meiosis are evolutionarily conserved and have been studied extensively (Zickler and Kleckner, 2015; Gray and Cohen, 2016; Hua and Liu, 2021; Chen et al., 2022). Errors in these crucial processes would lead to aneuploidy and genetic instability, so it is essential that they are monitored by a number of surveillance systems (Huang and Roig, 2023).

While spermatocytes carry out homologous recombination, they are also engaged in active transcription of a large number of genes that are essential for meiosis and spermiogenesis (Geisinger et al., 2021). To balance these two tasks, distinct chromatin domains are programmed during early prophase I to focus on either meiotic recombination or transcription activation (Alexander et al., 2023). Many meiosis genes start their expression at premeiotic stages and reach their peak levels at leptonema/zygonema, dropping at the zygonema/pachynema transition when the expression of late spermatogenesis genes rises sharply (Geisinger et al., 2021). Transcription of most genes on the sex chromosomes is silenced during meiosis as a result of asynapsis of the X and Y chromosomes beyond the pseudoautosomal regions; this process is known as meiotic sex chromosome inactivation (MSCI)

(Turner, 2015). MSCI can be viewed as an obligate form of a more generalized gene silencing mechanism known as meiotic silencing of unsynapsed chromatin (MSUC) that can occur on any chromosome when genes involved in synapsis are malfunctioning (Turner, 2015). Sex chromosome inactivation after meiosis is largely maintained in round spermatids via a heterochromatic compartment termed post-meiotic sex chromatin (PMSC), although some genes escape silencing for specific activation (Turner, 2015).

Sex chromosomes undergoing MSCI occupy a unique heterochromatic territory known as the sex body (or XY body) that is enriched with certain histone variants and histone modifications, as well as proteins involved in the DNA damage response (DDR), epigenetic modifications and transcriptional regulation (Li, 2023). MSCI starts with the phosphorylation of H2AX (γH2AX) by DDR proteins (including BRCA1, ATR, TOPBP1 and MDC1) at the chromosome axes (Alavattam et al., 2021). This phosphorylation extends across all the chromatin in the sex body via a feedforward mechanism called γH2AX-MDC1 signaling (Alavattam et al., 2021). Interestingly, it has been suggested that sex body formation and maintenance might occur through phase separation, which sequesters DDR factors from the autosomes to the sex chromosomes (Alavattam et al., 2021). However, further research is required to confirm this suggestion. MSCI serves many functions during spermatogenesis, including preventing DNA double-strand breaks (DSBs) from engaging in ectopic recombination, and it even has a profound effect on the development of the next generation (Ichijima et al., 2012). In pachytene spermatocytes and round spermatids, H3K27me3 is enriched on the TSSs of autosomal genes, but not the X-linked genes (Sin et al., 2015). These X-linked genes can be classified into MSCI-silenced genes and MSCI escape genes; MSCI-silenced genes maintain the suppressive marker H3K9me2 and activating markers H3K4me2/3, whereas the MSCI escape genes contain H3K9me2 but low levels of activating marker as early as in undifferentiated SG, suggesting that they are, unlike the autosomal late spermiogenesis genes, activated from the silent X chromosome but not poised for expression at the beginning of spermatogenesis (Sin et al., 2015). Although many regions that are open in SG are closed during MSCI, even more novel open regions are established in pachytene spermatocytes in sex chromosomes, which are later closed in round spermatids (Maezawa et al., 2018b).

Key regulators of meiosis that are specifically expressed in germ cells and loss of which results in meiotic arrest continue to be identified. They include transcription factors such as SCML2, ZFP541 and BEND2, which usually interact with other epigenetic modifiers to form transcription suppressive complexes (Hasegawa et al., 2015; Horisawa-Takada et al., 2021; Ma et al., 2022). These factors are essential for the suppression of somatic/progenitor genes and the activation of late spermatogenesis genes. SCML2 was first identified as a γH2AX-interacting protein in pachytene spermatocytes (Hasegawa et al., 2015). It interacts with RNF2, which is the catalytic core component of the PRC1 complex that suppresses gene expression by mono-ubiquitylating histone H2A at lysine 119 (H2AK119ub) in many cell types, including PGCs (Yokobayashi et al., 2013). In this context, SCML2 contributes to the establishment of H2AK119ub on autosomes, but prevents its deposition on sex chromosomes during meiosis (Luo et al., 2015; Hasegawa et al., 2015). *Zfp541* was first identified as a direct target gene of the STRA8-MEIOSIN complex; this complex plays a key role in the switch from mitosis to meiosis (Horisawa-Takada et al., 2021). *Bend2* is also a putative direct target of the STRA8-MEIOSIN complex, as indicated by our unpublished data (C.H., D. Xie). Both ZFP541 and BEND2 interact with HDAC1 and HDAC2 to modulate histone acetylation (Horisawa-Takada et al., 2021; Ma et al. 2022). N6-methyladenosine (m6A) is the most abundant mRNA modification, and it (alongside its writing, reading and erasing factors) has been shown to be crucial in spermatogenesis (Qian et al., 2022). Consistent with this, the key regulators of meiosis also include RNA-binding proteins, such as YTHDC2, MEIOC and RBM46, which together form a complex for downregulating mitotic transcripts (Qian et al., 2022). Meiosis in mice lacking these genes is initiated but cannot progress successfully and reaches an abnormal metaphase (Qian et al., 2022). Therefore, the meiotic state at early stages, such as leptonema and zygonema, is metastable and can go in the wrong direction if the mitotic gene program is not completely shut down. YTHDF2 also plays an essential role in the timely clearance of mRNAs in spermatocytes, probably in an m6A-dependent way (Qi et al., 2022). Whereas the complexes described above mainly function to shut down the mitotic program, some other factors function as activators of meiotic and post-meiotic programs. A-MYB (MYBL1) and TCL5 (TAL1) are transcription factors essential for exit from meiosis. *A-myb* is directly targeted by the STRA8-MEIOSIN complex, and A-MYB in turn activates the transcription of *Tcl5*, with A-MYB and TCL5 ultimately collaborating to activate the expression of a large number of meiotic and post-meiotic genes, including piRNA genes (Cecchini et al., 2023; Li et al., 2013; Maezawa et al., 2020).

Post-meiotic development of spermatids

Post-meiotic development of spermatids, also known as spermiogenesis, is characterized by dramatic morphological changes of spermatids, including the formation of special organelles, such as the flagellum and the acrosome, condensation of the nucleus and shedding of the majority of the cytoplasm. These morphological changes have been covered in detail in more specialized reviews (O'Donnell, 2014). There is a huge wave of transcription shortly after meiosis, and many transcription factors involved in this process have been identified (Wu et al., 2016; Zhang et al., 2018; Gustafson et al., 2020; Her et al., 2021; Tan et al., 2023). Genes expressed in spermatids are highly enriched on the X chromosome, suggesting that they are MSCI escape genes (Sin et al., 2015). The activation of these genes in spermatids is dependent on diverse proteins, including SCML2, RNF8 (an E3 ubiquitin ligase that promotes sex chromosome ubiquitylation) and CDYL (a major regulator of histone crotonylation); these factors collaborate to establish active epigenetic modifications (Adams et al., 2018). The chromatin remodeling that occurs during spermiogenesis is more dramatic than the chromatin remodeling that occurs at the other stages of spermatogenesis (Sassone-Corsi, 2002). Histones are replaced by protamines, resulting in DNA supercoils that are eliminated by DNA DSBs and repair; this process ultimately produces highly condensed sperm heads (Marcon and Boissonneault, 2004). Histone replacement is regulated by many factors/processes, including histone variants, histone hyperacetylation, transient DNA DSBs and repair, transition proteins and RNA-binding proteins (Hao et al., 2019; Tan et al., 2023). However, this replacement process is not fully completed, with about 1% of histones retained in mice and 15% in humans (Lismer and Kimmins, 2023). These retained histones are located at important regulatory genes involved in housekeeping, spermatogenesis and somatic development (Hammoud et al., 2009; Lismer and Kimmins, 2023). Genes for which promoters are simultaneously marked by H3K4me3 and H3K27me3 (so-called 'bivalent genes') in sperm overlap significantly with bivalent genes in embryonic stem cells and do not exhibit DNA methylation (Brykczynska et al., 2010). Phosphorylated Pol II and Mediator, a stabilizer of the transcription complex, colocalize to promoters in sperm, where they may either be poised to initiate gene expression after fertilization or serve as a memory of prior expression in spermatogenesis (Jung et al., 2019). Chromatin-binding proteins retained on the sperm genome that may be passed to the zygotes include CTCF, SMC1 and FOXA1 (Lismer and Kimmins, 2023).

Epidemiological studies and rodent models show that the harmful effects of paternal environmental exposures, including poor diet, toxicants and stress, can be transmitted to the next generations (Kahn et al., 2020). For example, a recent study shows that the bisphenol A-induced obesity epiphenotype can be transmitted to the F6 generation by epimutation in the CTCF binding site of the *Fto* gene (Jung et al., 2022). This histone retainment in sperm chromatin has led to the hypothesis that sperm histone codes are the mediators of intergenerational epigenetic information transmission (Gatewood et al., 1987; Gardiner-Garden et al., 1998). DNA methylation, non-coding RNAs and even transcription factors can also contribute to the paternal transmission of epigenetic information (Daxinger and Whitelaw, 2012; Lim and Brunet, 2013; Rando, 2016). The mechanisms by which environmental factors affect these transmitters are yet to be fully understood. For example, endocrine disruptors act as antagonists or agonists of the steroid hormone receptors, and trauma or stress induces the corticosterone response and a high-fat diet changes folate level; these intrinsic substances have all been shown to be potent modifiers of epigenetic information (Lismer and Kimmins, 2023), but how these environment-induced changes to the sperm epigenome are transmitted to the somatic and germline cells of the next generation to resist epigenetic reprogramming remains largely unknown.

Perspectives for future studies

Spermatogenesis is an interesting process that has been studied by researchers from different fields using multiple technologies, such as microscopic imaging, gene knockout and omic profiling, both *in vivo* and *in vitro*. These approaches are beginning to uncover the dynamic changes to cellular activity, gene expression and the epigenetic landscape that take place during spermatogenesis, but how these processes are orchestrated to occur in an orderly manner is still poorly understood. This is mainly due to the fact that any single event is controlled by a huge number of genes that interact to form an astonishingly complex regulatory network. Our lack of in-depth understanding of spermatogenesis is reflected by our inability to replicate this process completely *in vitro*, despite some recent progress (Lei et al., 2021). Moreover, most of our knowledge about spermatogenesis is based on studies using the mouse as a model organism and we know little about other species. The good news is that more and more powerful tools are now in our hands. CRISPR-based functional screening of germ cells from different species (both *in vivo* and *in vitro*) followed by single-cell omics studies and deep learning-guided construction of gene networks will push our understanding of spermatogenesis to a significantly higher level. This understanding will in turn contribute to elucidating the general rules governing the germline cycle and somatic development.

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I apologise that many excellent research papers are not referenced owing to space limitations.

Competing interests

The author declares no competing or financial interests.

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High-resolution poster

A high-resolution version of the poster is available for downloading at https://journals.biologists.com/dev/article-lookup/doi/10.1242/dev.202033#supplementary-data

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