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Epigenetic priming in the male germline

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Abstract

Epigenetic priming presets chromatin states that allow the rapid induction of gene expression programs in response to differentiation cues. In the germline, it provides the blueprint for sexually dimorphic unidirectional differentiation. In this review, we focus on epigenetic priming in the mammalian male germline and discuss how cellular memories are regulated and inherited to the next generation. During spermatogenesis, epigenetic priming predetermines cellular memories that ensure the lifelong maintenance of spermatogonial stem cells and their subsequent commitment to meiosis and to the production of haploid sperm. The paternal chromatin state is also essential for the recovery of totipotency after fertilization and contributes to paternal epigenetic inheritance. Thus, epigenetic priming establishes stable but reversible chromatin states during spermatogenesis and enables epigenetic inheritance and reprogramming in the next generation.

Introduction

The germline is the only cellular lineage capable of transmitting genetic and epigenetic information to the next generation. In the mammalian germline, primordial germ cells (PGCs), the precursors of sperm and eggs, are specified in the early embryos and undergo epigenetic reprogramming to reset previous epigenetic states [1]. Upon migration to the gonads, PGCs receive sex-specific signals from the surrounding somatic cells, which initiate either spermatogenesis or oogenesis [2]. After reaching the gonad, male germ cells are called prospermatogonia (also known as gonocytes) and arrest at the G1/G0 phase of the cell cycle. Prospermatogonia later resume the cell cycle after birth, and a subset of them convert to spermatogonial stem cells (SSCs), which sustain the lifelong production of sperm [3]. After the commitment to differentiation, male germ cells undergo meiosis and produce haploid sperm (Figure 1) [4]. Although single-cell RNA-seq analysis revealed various substages and transitions during spermatogenesis [5], the final cell fate of male germ cells after sex determination is sperm unless the cells undergo cell death [6]. Consistent with this unidirectionality of the differentiation process, recent studies revealed that gene expression programs in various stages of spermatogenesis are predetermined at the chromatin level

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during prior stages of development [7–10] (Figure 2). This suggests that chromatin-based cellular memories ensure the unidirectional differentiation process during spermatogenesis.

Another major aspect of epigenetic regulation in the germline is the preparation for embryonic development in the next generation. Several studies demonstrate that the chromatin state of sperm contributes to the transmission of epigenetic information to the offspring [11–13]. Epigenomic instability in sperm is associated with an increased risk of abnormal embryogenesis, highlighting the importance of the paternal epigenome in embryonic development [11,14].

Epigenetic priming, observed in a variety of cell types such as pluripotent stem cells, neurons, immune cells, and cancer cells, presets chromatin states that enable the induction of gene expression programs in response to differentiation cues [15–18]. In the male germline, epigenetic priming provides stable but reversible chromatin states that guide unidirectional spermatogenesis and allow subsequent epigenetic inheritance and reprogramming in the next generation. In this review, we discuss recent findings that highlight the importance of epigenetic priming for autosomal gene expression programs during the major stages of male germline development, as well as for the subsequent epigenetic inheritance and reprogramming in the next generation. For other key aspects of germline development, such as the regulation of the sex chromosomes, retrotransposons, and gene regulation in the female germline, we refer readers to recent reviews on these topics [3,19–22].

Epigenetic reprogramming in primordial germ cells

Epigenetic reprogramming in PGCs provides the foundation for subsequent gametogenesis by resetting the prior epigenetic state. In mice, extraembryonic signals trigger the emergence of PGCs from pluripotent epiblast cells during gastrulation. PGCs then migrate to the genital ridges, which give rise to the gonads. During this period, sequential global and locus-specific DNA demethylation takes place. First, global methylation levels are reduced through rounds of DNA replication; subsequently, from embryonic day 9.5 (E9.5) to E13.5, methylated cytosines at imprinted control regions and meiotic genes are actively demethylated by ten-eleven translocation (TET) methylcytosine dioxygenase [23]. After DNA demethylation, meiotic genes are suppressed through repressive chromatin modifications mediated by Polycomb Repressive Complex 2 (PRC2), which trimethylates histone H3 at lysine 27 (H3K27me3) [24,25]. Interestingly, a subset of germline gene promoters are modified with H3K27me3 at the epiblast stage, and these H3K27me3 marks appear to persist into PGCs, suggesting that the epigenetic state persists, at least in part, from the epiblast stage into PGCs [24]. PRC2 may also be involved in the epigenetic priming of a broader range of target genes. In human hypomethylated male PGCs between weeks 7 and 9, corresponding to E13.5 in mice PGCs in terms of DNA methylation level, 76% of male-specific H3K27me3-marked promoters are also modified with an active promoter mark H3K4me3 [26]. These types of bivalent promoters are indicative of a transcriptionally poised epigenetic state [15]. Thus, these results suggest that DNA demethylation and subsequent PRC2-dependent gene regulation are key aspects of epigenetic priming in PGCs.

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Epigenetic programming in prospermatogonia

In mice, after E13.5, male PGCs, called prospermatogonia, proliferate and then enter G1/G0 cell cycle arrest. Subsequently, prospermatogonia either undergo the first wave of spermatogenesis [27] or give rise to a pool of foundational SSCs. This process is mediated by the germ cell–specific X-linked homeobox transcription factor RHOX10, which acts in concert with the downstream transcription factors DMRT1 and PLZF to generate SSCs [28,29]. RHOX13, another X-linked homeobox transcription factor, is required for the first wave progression but not for differentiation into SSCs [30]. H3K9 demethylases, JMJD1A/B, demethylate H3K9me2 in prospermatogonia and regulate the SSC maintenance genes in the subsequent undifferentiated spermatogonia [31], supporting the notion that prospermatogonia are epigenetically primed to generate SSCs.

Importantly, during the prospermatogonia stages, there is a global increase in DNA methylation relative to the basal level in PGCs. In fact, in mice, the overall DNA methylation levels in prospermatogonia at postnatal day 0.5 (P0.5) are similar to that of sperm, except for promoter and enhancer regions of a small number of stage-specific genes [32]. Before the establishment of DNA methylation, there is a genome-wide gain of accessible chromatin [33]. Depletion of a chromatin remodeler SNF5 in male germ cells affects DNA methylation and cell cycle arrest, indicating that SNF5 is important for the chromatin states necessary for epigenetic programming in prospermatogonia [34]. To initiate de novo DNA methylation, the histone methyltransferase NSD1 mediates H3K36 dimethylation (H3K36me2) at genomic regions that will gain DNA methylation. The PWWP domain of the *de novo* DNA methyltransferase DNMT3A recognizes H3K36me2, leading to methylation of these regions [35]. DNMT3A-deficient prospermatogonia give rise to self-renewing SSCs, but these SSCs cannot commit to spermatogonial differentiation, suggesting that DNA methylation acquired in prospermatogonia is required for spermatogenic gene expression [36]. Of note, early prospermatogonia have a left-handed Z-DNA structure in part of the genome, and a zinc finger protein ZBTB43 resolves Z-DNA to induce *de novo* DNA methylation [37]. Concomitant with the gain of DNA methylation, gene expression patterns change substantially around E16.5 [38]. A recent study established *in vitro* reconstituted mouse spermatogenesis, which corresponds to E11.5 to P5~7, and suggested that the acquisition of the androgenic epigenome takes place during the cell cycle arrested phase of prospermatogonia in fetal testes [38], which coincides with the establishment of genome-wide DNA methylation [35]. These molecular events suggest that global gain of DNA methylation is a part of the mechanisms of epigenetic priming for spermatogonial differentiation.

Epigenetic priming for stem cell maintenance and differentiation in spermatogonia

Mammalian males sustain lifelong fertility by balancing self-renewal and differentiation of SSCs. SSC activity resides in a heterogenous population of undifferentiated spermatogonia, whose cell states are maintained at a delicate equilibrium [39]. Within this population, epigenetic regulation performs two major functions: stem cell maintenance

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and preprogramming of spermatogenic differentiation. In addition, epigenetic priming is implicated in establishing the foundational SSC pool [40].

SSC maintenance is regulated by epigenetic regulators such as PRCs [41–43], the H3K79 histone methyltransferase DOT1L [44], and a chromatin remodeler CHD8 [45]. PRCs regulate bivalent domains marked with H3K27me3 and H3K4me3, a signature of a primed state that is extensive in germ cells throughout their development, including juvenile and adult undifferentiated spermatogonia [8,9,46–50]. Our recent study demonstrated that PRC1 shields adult undifferentiated spermatogonia from differentiation, maintains slow cycling, and directs commitment to differentiation during steady-state spermatogenesis in adults [51]. We show that PRC1 directs PRC2-mediated H3K27me3 as an epigenetic hallmark of adult undifferentiated spermatogonia. Spermatogonial differentiation is accompanied by a global loss of H3K27me3 from genes required for the process [51]. PRC2 is also critical for later stages of spermatogenic differentiation because meiotic entry is affected in PRC2-depleted male germ cells [43].

Spermatogenic differentiation is preprogrammed at the chromatin level. In undifferentiated spermatogonia, SCML2, a germline-specific component of PRC1, binds to thousands of active genes with hypomethylated promoters that are enriched with H3K4me3 [7,8], a mark set by the histone methyltransferase KMT2B (also known as MLL2) [52]. SCML2 induces PRC2-mediated H3K27me3 on these target genes in the meiotic prophase, establishing extensive bivalent domains and leading to global suppression of these genes [8]. The germ cell–specific protein BEND2 was recently identified as a meiotic regulator that restrains H3K4me3 levels in zygotene spermatocytes, and the depletion of BEND2 increases H3K4me3 level (Figure 2a) [53]. Although the molecular function of BEND2 is unknown, its homolog BEND3 prevents the premature activation of genes with bivalent domains during embryonic stem (ES) cell differentiation [54,55]. Notably, BEND2 interacts with SCML2, and the *Bend2* gene locus is next to the *Scml2* gene locus on the X chromosome; thus, BEND2 and SCML2 may be coregulated to work together to establish bivalent domains. Curiously, KMT2B is also implicated in PGC specification [56], raising the possibility that regulation of H3K4me3 is critical for an acquisition of the germline potential.

In addition to the preprogramming of gene repression, gene activation is preprogrammed as well. In cultured germline stem (GS) cells, which are a proxy for undifferentiated spermatogonia, RNA polymerase II (Pol II) and the active H3K4me2 mark are present at promoters of suppressed genes that are to be activated in meiotic prophase [9]. Accumulation of H3K4me2 at these promoters was confirmed in undifferentiated spermatogonia [8]. These meiotic prophase genes are suppressed by PRC1.6, a subcomplex of PRC1, in ES cells [57,58], but the role of PRC1.6 in the regulation of meiotic prophase genes in the male germline *in vivo* remains to be determined. When spermatogenic differentiation is induced and the cells enter meiosis, these genes are upregulated by the transcription factors STRA8 and MEIOSIN [59,60]. Expression of *Meiosin* is facilitated by the deposition of the histone variant H2A.Z at the *Meiosin* gene locus, mediated by a chromatin remodeler ZNHIT1. ZNHIT1 also regulates meiotic prophase genes downstream of STRA8 and MEIOSIN [61]. Curiously, in early embryos and ES cells

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where their meiotic prophase gene promoters are hypomethylated, PRC1.6 recruits the H3K9 methyltransferase SETDB1 and represses the meiotic prophase genes by depositing H3K9me3 [62], uncovering the layers of regulatory mechanisms of meiotic prophase genes.

Taken together, these observations show that epigenetic priming is evident in undifferentiated spermatogonia. Spermatogenic differentiation programs are predetermined at the chromatin level before the onset of differentiation akin to a lunch box, which contains a preprepared meal (Figure 2d).

Transcriptional burst of meiotic genes in pachytene spermatocytes

After entering meiosis, sequential cascades of gene activation take place. Genes that are upregulated in the preleptotene stages are required for the events in early meiotic prophase, while genes whose transcription is activated in the later pachytene stage mainly function in postmeiotic stages. Concomitant with the transcriptional burst that occurs at the pachytene stage, the chromatin state of the differentiating cells changes dynamically, including accessible chromatin and histone modifications [8–10,63]. The transcription factor A-MYB (MYBL1), which is expressed in the meiotic prophase, acts as a master regulator of the pachytene transcriptional burst [64]. It activates various loci, including pachytene Piwiinteracting RNA (piRNA) precursor loci [65], meiosis-specific super-enhancers (SEs) [10], and retrotransposon-derived enhancers [66]. As briefly discussed above, meiotic SEs are poised with H3K4me2 in spermatogonia (Figure 2b) [10], indicating that epigenetic priming with H3K4me2 presets later gene activation. Before the pachytene stage, Pol II is already loaded to the promoters of the A-MYB target genes in the leptotene and zygotene stages of the early meiotic prophase. At the later pachytene stage, A-MYB and the testis-specific bromodomain protein BRDT are loaded to activate the quiescent Pol II and trigger the transcriptional activation [67]. Consistent with this observation, Pol II pausing is shown to be essential for appropriate gene expression during spermatogenesis [68] The testis-specific transcription factor TCFL5 responds to A-MYB and forms a feedback loop with A-MYB [69]; thus, A-MYB drives a transcriptional network to coordinate the burst of pachytene transcription. Of note, ATF7IP2 (also known as MCAF2), a germline-specific partner of SETDB1, regulates H3K9me3 in meiotic prophase but also, counter-intuitively, binds and directly activates a large number of pachytene-activated genes [70]. On the other hand, a transcription factor ZFP541 suppresses a part of meiotic prophase genes to promote meiotic prophase exits [71–73]. However, it is unknown how or if these factors collaborate and how these gene regulatory mechanisms are coordinated to ensure meiotic progression.

Epigenetic priming of the male epigenome for the next generation

During the final stages of spermatogenesis, the chromatin states that are established at the pachytene stage are progressively remodeled to form condensed sperm nuclei [74]. Most histones are replaced with protamines, although a small portion of histones remain (1–8% in mice and 10–15% in humans) [75–79]. In mouse and human sperm, bivalent marks (H3K4me3 and H3K27me3) persist on gene promoters that are activated later in embryogenesis, suggesting that embryonic gene expression programs are epigenetically primed in the paternal epigenome before fertilization [80]. Several studies have examined

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the functional significance of paternally derived H3K4me3 and H3K27me3 for embryonic development in the next generation. Over-expression of the histone H3K4 demethylase KDM1A in developing sperm impairs embryonic development in F1 (but F1 mice are viable), and the defect persists into F3 [11,14]. Although this phenotype appears to be independent of bivalent domains [81], H3K4me3 persists from sperm to early embryo to regulate gene expression [14]. Other studies showed that paternal deletion of the H3K27me3 demethylase KDM6A disturbs spermatogenic gene expression, leading to abnormal epigenetic inheritance and increased cancer susceptibility in the next generation [82,83]. In addition, reduction of H3K27me3 levels in testicular sperm through *Scml2* deletion causes abnormal gene expression in the next generation without the transmission of the mutant allele (because the X-linked *Scml2* gene allele is not transmitted to male offspring), demonstrating SCML2-mediated epigenetic inheritance [13]. Thus, *Scml2*-KO mice are a good model for studying epigenetic inheritance from the paternal germline because all male pups sired from *Scml2*-KO males are genetically wild type. A recent study compared embryos derived from intracytoplasmic sperm injection using epididymal sperm and round spermatid injection using round spermatids and reported that paternal H3K27me3 is linked to gene expression changes in the early embryo [84]. These studies suggest that paternal H3K27me3 mediates intergenerational epigenetic inheritance. The underlying molecular mechanisms remain unknown, in part because the paternally derived histone variant H3.3, a major histone in sperm, is replaced in zygotes [85], and therefore, paternal H3K27me3 is erased in the early embryo [86]. Key outstanding questions in the field currently include to what extent the paternal epigenome contributes to embryonic development and how paternal epigenetic inheritance withstands the extensive remodeling of paternal chromatin that takes place after fertilization.

3D chromatin structure in spermatogenesis

The application of genome-wide chromosome conformation capture methods during spermatogenesis has begun to provide insight into 3D genome organization during spermatogenesis [87–91]. Male germ cells undergo extensive remodeling of the 3D chromatin organization during meiotic prophase I [87,92] (Figure 2c). The number of chromatin loops and topologically associated domains (TADs) is reduced in pachytene spermatocytes compared with spermatogonia stages. Nevertheless, some persist into mature sperm, and their anchor sites and TAD boundaries tend to be modified by active (H3K4me3 and H3K27ac) and repressive (H3K27me3) marks [87]. This raises the possibility that 3D chromatin carries cellular memories of gene expression programs in the male germline. Of note, GS cells have largely attenuated 3D chromatin features and distinct CCCTC-binding factor (CTCF) distributions compared with induced PGC-like cells, which represent PGCs [93]. This suggests that acquisition of the androgenic program after the PGC stage is accompanied by 3D chromatin remodeling. Our recent study shows that CTCF-mediated 3D chromatin predetermines the gene expression program required for spermatogenesis [94]. In undifferentiated spermatogonia, CTCF-mediated chromatin contacts on autosomes pre-establish meiosis-specific super-enhancers, suggesting that CTCF-mediated 3D chromatin organization enforces epigenetic priming that directs unidirectional differentiation. These

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results suggest that epigenetic priming during spermatogenesis involves 3D genome reorganization.

Are 3D chromatin structures passed on to the next generation? Do they contribute to the regulation of gene expression during embryogenesis? In the one-cell embryos, the paternal genome carries specific chromatin loops, which persist into the inner cell mass. Of note, these loops are already evident in sperm [95]. In the maternal genome, these loops are not detected until the eight-cell stage, suggesting the persistence of these loops from sperm to zygote. Furthermore, the inheritance of sperm chromatin structure has been examined in a mouse model in which pregnant female mice are exposed to bisphenol A (BPA), a compound mimicking estrogen [96]. BPA exposure altered chromatin accessibility at CTCF-binding sites near the *Fto* gene in sperm over six generations, and this was correlated with the transmission of an obesity phenotype. Thus, it is tempting to speculate that paternal 3D chromatin structures could be transmitted transgenerationally, despite extensive remodeling of paternal chromatin. Nevertheless, a recent study raised possible technical issues in interpreting sperm Hi-C data [97], and ongoing debates continue in the field.

Perspectives

Akin to a lunch box, into which meals, snacks, and beverages are packed in the morning for use later in the day, epigenetic priming prepares future gene expression programs for later activation upon differentiation cues. During spermatogenesis, it ensures unidirectional differentiation to sperm and subsequent embryogenesis upon fertilization (Figure 2d). However, how epigenetic priming is initiated and how chromatin-based memories function to define male germ cell identity is currently unknown. To address these questions, key mechanisms surrounding chromatin regulation must be examined. Such mechanisms include extracellular and intracellular signaling pathways that direct male germ cell differentiation, transcription factor networks, and post-transcriptional regulation at the RNA level. For example, various germline-specific RNA-binding proteins determine germline identity [98], but it is unclear if and how epigenetic gene regulation interacts with post-transcriptional regulation to define cellular phenotypes. Further technical advances in next-generation sequencing-based methods, including single-cell analysis, will facilitate a more detailed study of epigenomic features and the heterogeneity between individual germ cells.

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Data Availability

No data were used for the research described in the article.

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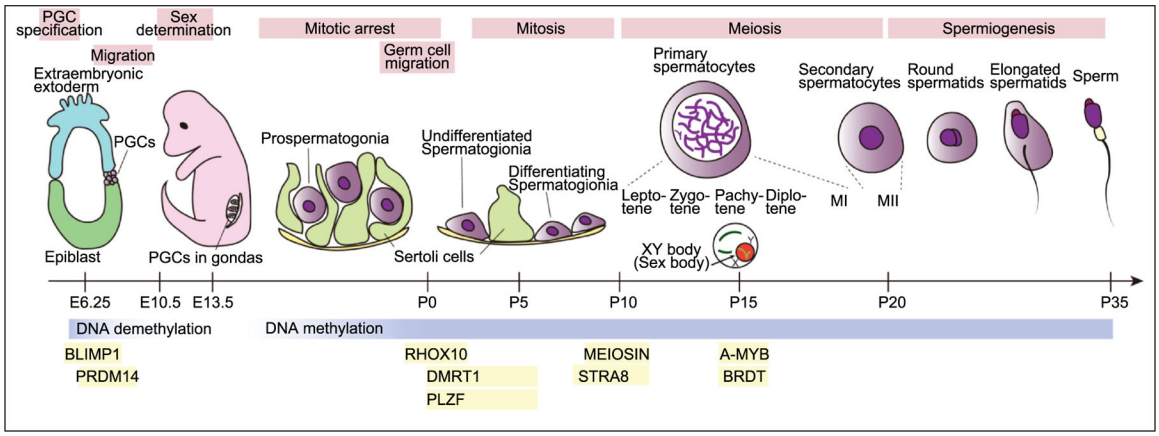

Figure 1.

The overview of male germline development in mice. PGCs emerge from the epiblast at E6.25. During their subsequent migration to the gonads, global DNA demethylation takes place. DNA *de novo* methylation and mitotic arrest occur approximately from E13.5 to P0.5. After birth, prospermatogonia either undergo the first wave of spermatogenesis or give rise to a pool of foundational SSCs. Undifferentiated spermatogonia differentiate into differentiating spermatogonia in response to retinoic acid, and subsequently, they initiate meiosis and undergo two cell divisions, ultimately forming haploid sperm. Key transcriptional regulators are indicated at the bottom.

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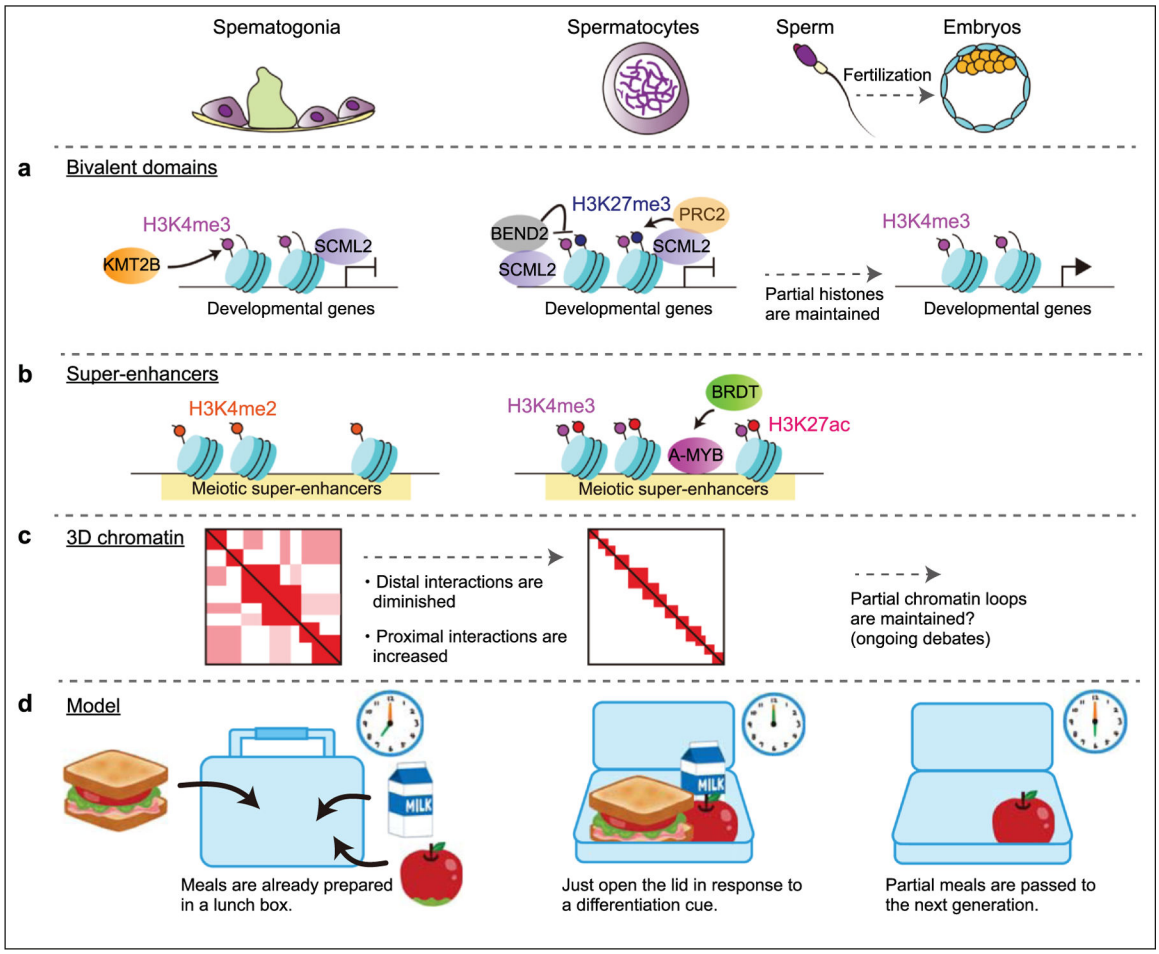

Figure 2.

Epigenetic priming in the male germline: the lunch box model. **(a)** Bivalent domains marked with both H3K4me2/3 and H3K27me3 are formed at promoters of embryonic developmental genes suppressed throughout the germline and somatic genes suppressed during late spermatogenesis. **(b)** Meiotic SEs, which facilitate the transcriptional burst in pachytene spermatocytes, are formed by A-MYB. **(c)** Alterations in 3D chromatin structure during spermatogenesis. **(d)** The lunch box model of epigenetic priming in spermatogenesis. The differentiation program of late spermatogenesis (especially the burst of gene expression in pachytene spermatocytes) is preset at the chromatin level in spermatogonia. This is akin to the meal that is already prepared in the lunch box. Once differentiation cues are received, differentiation is initiated. The lunch box lid is opened. Gene expression after fertilization is also preprogrammed in the germline at the chromatin level. The copyright of the illustration is attributed to Takashi Mifune (https://www.irasutoya.com/).