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Visions & Reflections (Minireview)

Epigenetic regulation for the induction of meiosis

Y. Matsui[a, b, *] and K. Hayashi[c]

[a] Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University, Seiryo-machi 4-1, Sendai 980-8575 (Japan), Fax: +81 22 717 8573, e-mail: ymatsui@idac.tohoku.ac.jp
[b] CREST, JST (Japan Science and Technology Agency), Saitama (Japan)
[c] Wellcome Trust/Cancer Research UK Gurdon Institute of Cancer and Developmental Biology, University of Cambridge, Cambridge (UK)

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Abstract. The germ cell lineage is the sole cell lineage through which genomic information is transmitted into successive generations. To this end, germ cells undergo various specific differentiation steps including meiosis, whose regulation seems to correlate closely with fundamental mechanisms that create differences between germ cells and somatic cells. In mammals, meiosis is triggered by extra-embryonic stimuli such as retinoic acid, which may induce various intracellular molecular cascades promoting meiosis. In addition, the specific epigenetic status arranged in germ cells before and after induction of meiosis, including meiosis-specific transcription control based on histone methylation by a novel histone methyltransferase Meisetz, is also critical for its proper progression.

Keywords. Germ cell, meiosis, histone methylation, DNA methylation, methyltransferase, retinoic acid, chromatin, chromosome.

Induction of meiosis in mice

Meiosis is the highly specialized cell division that is essential for germ cells to develop into functional haploid gametes. Despite the biological importance of meiosis, little is known about the regulation of the initiation and the early progression of meiosis, especially in mammals. The mechanisms of meiotic initiation have been partly explained in yeast [1, 2], and various extracellular stimuli are known to be critical for regulation of mitosis-meiosis switching in yeast, and in higher organisms such as nematodes [3–6].

In mammals, previous observations have suggested that putative environmental cues that inhibit meiosis play important roles [7], but the molecular nature of these processes remains obscure. In embryonic mouse gonads, fetal germ cells proliferate mitotically until mid-gestation. Male germ cells subsequently undergo mitotic cessation after E (embryonic day) 13.5. They resume mitosis, develop into spermatogonia and initiate meiosis after birth. In contrast, female germ cells immediately enter meiotic prophase at E13.5. A number of experimental findings have suggested that even fetal male germ cells are ready to initiate meiosis in embryos, but that the environment in fetal testes prevents germ cells from entering meiosis. For example, male germ cells do not normally enter meiosis in embryonic gonads, but those ectopically located outside gonads, for example, in the adrenal gland, initiate meiosis [8]. In addition, male germ cells at E11.5 do not enter meiosis in cultured intact gonads as in the embryo, but if the germ cells are purified and cultured after reaggregation with fetal lung cells, they can enter meiosis [9]. Although the roles of meiosis inhibitory substances are likely important in determining the timing of meiosis, it was unclear whether meiosis is cell-autonomously initiated or induced by a putative meiosis-inducing substance

* Corresponding author.

that most likely exists in a wide variety of tissues, including fetal lung and adrenal gland. Recent studies have finally clarified the nature of meiosis induction, as well as the role of inhibiting substances [10, 11].

The two research groups involved have shown that retinoic acid (RA) functions as an inducer of meiosis in mouse embryonic gonads. RA induces the expression of meiosis-specific genes such as Stra8, Scp3 and Dmc1, while its antagonist suppresses their expression as well as the appearance of histologically distinct meiotic germ cells in cultured embryonic gonads [10, 11]. Genes induced by RA would most likely trigger meiosis, and Stra8 was suggested to be one such gene [10]. RA is produced in mesonephros adjacent to embryonic gonads in both males and females and then diffuses into the gonads [11]. However, in embryonic testis, Sertoli cells in testicular tubules express the retinoid-degrading enzyme CYP26B1. An antagonist of the enzyme induces the expression of meiotic marker genes and the chromosome arrangement specific for meiosis in cultured embryonic testis, and meiotic genes are induced in embryonic testis of Cyp26b1-null mice [10, 11]. These findings indicate that Cyp26B1 in embryonic testis removes RA, and, therefore, embryonic male germ cells do not enter meiosis (Fig. 1). On the other hand, embryonic ovary does not express CYP26B1 and therefore is exposed to high doses of RA to undergo meiosis in the germ cells. Although RA is certainly one of the most proximal inducers of meiosis in mice, it is still not fully clear whether RA evokes the signal directly in the germ cells, and further studies will uncover signaling cascades activated by RA in germ cells and possibly in the supporting somatic cells as well.

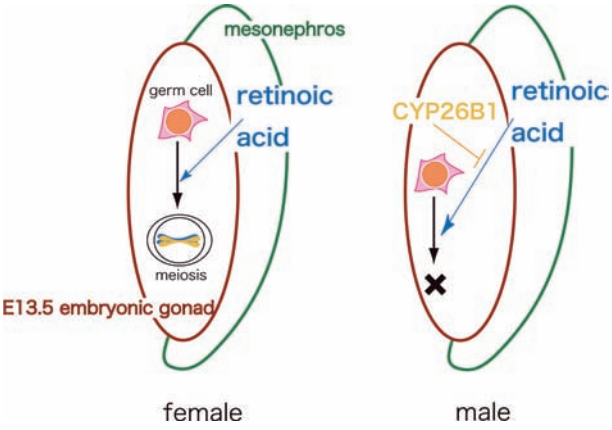

Figure 1. Retinoic acid produced in mesonephros induces meiosis in female embryonic gonads. In male embryonic gonads, the retinoid-degrading enzyme, CYP26B1, expressed in Sertoli cells removes retinoic acid and thus germ cells do not enter meiosis.

Regulation of meiotic progression by DNA methylation and the small RNA pathways

Although RA is a key regulator of meiosis, germ cells may require numerous factors and events to be in alignment at pre-meiotic stages to initiate meiosis, and recent studies have indicated the importance of germ cell-specific epigenetic status for the initiation and early progression of meiosis.

Homozygous mutations of genes encoding a *de novo* DNA methyltransferase Dnmt3a [12] or its associated molecule Dnmt3L [13, 14] cause a deficiency in meiosis in testis. In Dmnt3L homozygous mutant mice, pairing of homologous chromosomes during meiotic prophase is severely impaired and meiosis is arrested at the pachytene spermatocyte stage. Interestingly, DNA methylation of non-pericentric heterochromatic regions and of interspersed repeats, including retrotransposons, does not occur in the mutant testicular germ cells. Consistent with the DNA hypomethylation in the sequences, expression of retrotransposons is elevated in Dnmt3L homozygous testis [13], indicating that although testis is known as a tissue preferentially expressing retrotransposons [15, 16], chaotic expression of the retrotransposons causes defects in meiosis.

Linkage between the small RNA pathway and gene silencing has been demonstrated, and these studies have indicated that this pathway is also critical for repression of repetitive sequences in the germline and for meiotic progression. Argonaute family genes play important roles in RNA silencing and translational regulation in various organisms [17]. In *Drosophila* testes, 24- to 29-nucleotide rasi (repeat-associated small interfering) RNAs which are slightly longer than conventional miRNA (21–22 nucleotides) are produced from *Suppressor of Stellate* [*Su(Ste)*] tandem repeats and mediate silencing of the *Stellate* repeat as well as LTR and non-LTR retrotransposons through binding with Piwi or Aubergine, members of the Argonaute family [18, 19]. Deletion of the *Su(Ste)* repeat results in hyper-expression of *Stellate* in testes and causes meiotic abnormalities [20]. In addition, mutations in *Drosophila piwi*, *aubergine* or *homeless* caused loss of silencing of transgenes and reduction of histone H3K9 methylation as well as delocalization of HP1 and HP2, critical components of heterochrimatin [21]. Other studies have also shown that RNAi machineries in *Saccharomyces pombe* [22, 23] and *Arabidopsis* [24] are involved in heterochromatin assembly at least in part via DNA and histone H3K9 methylation. Those results indicate that the small RNA pathway is involved in heterochromatin assembly and gene silencing, and probably regulates meiosis via silencing repetitive sequences by chromatin modifications. In mouse testis, *Miwi* and *Mili*, homologues of *piwi*, are essential for spermatogenesis. Deficiencies in the *Miwi* and *Mili* genes cause spermatogenic arrest in round spermatids and pachytene spermatocytes, respectively [25, 26]. More recently, novel, germline-specific

small RNAs, piRNA (PIWI-interacting RNA), which, like rasi RNAs, are longer (26–31 nucleotides) than conventional miRNAs, were detected, and they bind to Miwi and Mili [27, 28]. The detailed mechanisms by which meiosis is regulated by the small RNA pathways are quite interesting and need to be clarified further.

In addition to the regulation of functions of retrotransposons, methylation of such sequences may lead to heterochromatinization throughout the genome. During the early stages of meiotic prophase, dimethylation of histone H3 lysine 9 (K9), involved in heterochromatin formation, decreases earlier in Dnmt3L-deficient spermatocytes than in wild-type spermatocytes, while acetylation of histone H3K9 and of histone H4, both of which cause euchromatinization, is maintained at high levels until later stages in the mutant spermatocytes [13]. This observation suggests that Dmnt3L is involved in the maintenance of heterochromatin during early meiotic prophase. It has been suggested that compaction of meiotic chromosome structure in early spermatocytes facilitates chromosomes to move more freely and to find homologous partners [29]. Although further direct evidence is currently not available, these results suggest that methylation of non-pericentric heterochromatic regions and of interspersed repeats by Dmnt3L may lead to compaction of chromosome structure, which may be essential for proper alignment of homologous chromosomes (Fig. 2).

Notably, although DNA methylation by Dnmt3L is definitely involved in meiotic progression, its expression is restricted in pre-meiotic germ cells during a short perinatal period [13, 14]. Because meiosis in male germ cells is first initiated at about 10 days after birth and occurs continuously throughout the life of the individual, the perinatal-specific expression of Dnmt3L indicates that the establishment of specific chromatin structure long before the initiation of meiosis is necessary for proper progression of meiotic prophase.

Regulation of meiosis by histone H3K9 methyltransferases.

Histone H3K9 methylation also controls the behavior of meiotic chromosomes [30]. A histone H3K9 methyltransferase, Suv39h1, is expressed in many tissues, while Suv39h2 is specifically expressed in testis [31]. Its expression is detectable only in type B spermatogonia that will soon start meiosis and in pre-leptotene spermatocytes just initiating meiosis. Although mice with deletions of either the Suv39h1 or Suv39h2 genes are normal, mutation of both genes causes non-homologous interactions and delayed synapsis of meiotic chromosomes [30]. In these mutant mice, pericentric histone H3K9 methylation is impaired just before and during early meiotic prophase.

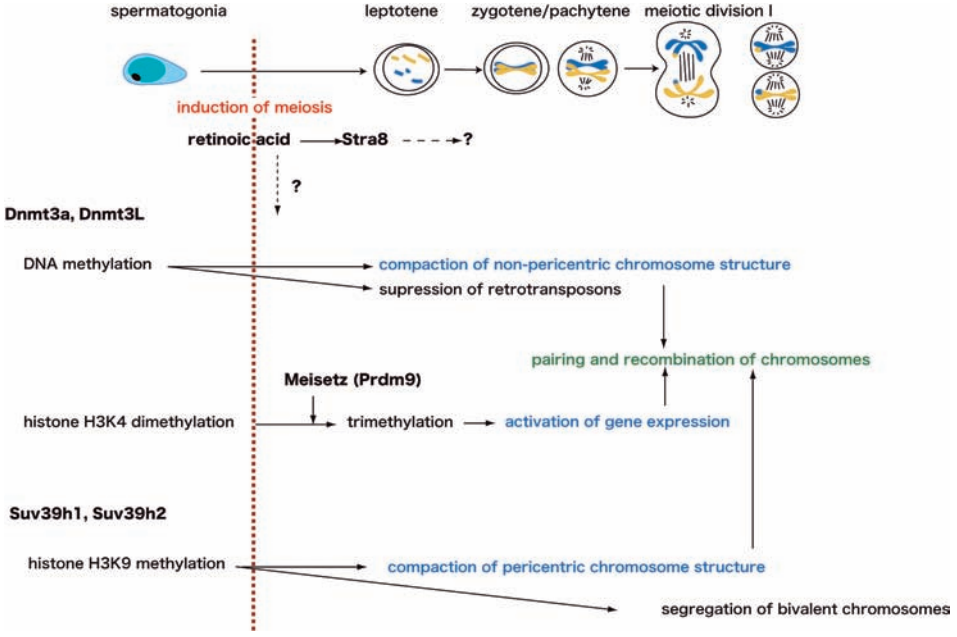

Figure 2. Retinoic acid plays a critical role in the initiation of meiosis in mice. Retinoic acid induces the expression of Stra8, which may regulate subsequent molecular cascades including specific epigenetic changes required for meiotic progression, but the detailed functions of Stra8 are unknown. Methylation of non-pericentric heterochromatic sequences and of retrotransposons by Dnmt3a and Dnmt3L, and pericentric histone H3K9 methylation by Suv39h, may be involved in compaction of chromosome structure as well as suppression of retrotransposons, both of which may be essential for proper association of homologous chromosomes. These methylations occur, at least in part, before induction of meiosis. Histone H3K4 trimethyltransferase, Meisetz, activates genes required for pairing and recombination of homologous chromosomes. Because Meisetz is a trimethylation-specific enzyme, histone H3K4 around the required genes is most likely dimethylated before meiosis.

Because histone H3K9 methylation also leads to heterochromatinization, these findings suggest that compaction of chromatin around the centromere is also important for pairing of homologous chromosomes (Fig. 2). The expression of Suv39h and histone H3K9 methylation occurs before meiosis [30, 31], and this suggests that specific changes in pericentric chromatin structure caused by Suv39h may be a premise for proper progression of meiotic prophase.

Meiosis-specific transcriptional control by a histone H3K4 trimethyltransferase, Meisetz

Recent studies have revealed that histone modification and DNA methylation regulate the accessibility of transcription factors to proper gene loci [32–35], leading to the possibility that changes in germ cell-specific patterns of epigenetic status may critically control gene expression, which may restrict meiosis in germ cells. In fetal germ cells before entering meiosis, a low level of dimethylated histone H3K9 and a high level of trimethylated histone H3K27 are observed [36]. Similar patterns of histone modification are also observed in spermatogonia [37], and thus these patterns seem to be maintained before entering meiosis. However, once germ cells enter meiosis, dimethylated histone H3K9 is up-regulated in spermatocytes [37] as well as fetal female germ cells after E13.5 [our unpublished data], whereas the level of trimethylated histone H3K27 is not altered. In addition to histone H3K9 and K27 methylation, trimethylation of histone H3K4 is up-regulated in pachytene spermatocytes [38]. Therefore, it is most likely that these changes in histone modifications may define a chromatin status required for meiosis-specific gene expression.

We recently provided evidence for a functional linkage between a specific histone modification and meiosis-specific gene expression. We identified a novel histone methyltransferase, Meisetz (Prdm9), which plays an essential role in meiosis [38]. Meisetz is specifically expressed in early meiotic germ cells both in testis and ovary. It has a PR domain that is responsible for trimethyltransferase activity specific to histone H3K4, and this activity leads to transcriptional activation. Meisetz also contains a C2H2 zinc finger in its C-terminal region, which is involved in nuclear localization of the protein. Analysis of Meisetz-null mice revealed that Meisetz is necessary for pairing and recombination of homologous chromosomes during meiotic prophase [38]. In Meisetz-deficient spermatocytes, histone H3K4 trimethylation is decreased throughout the genome, and the expression of a number of autosomal genes, including those specifically expressed in meiotic germ cells, is suppressed. These findings suggest that Meisetz may trimethylate histone H3K4 around a set of genes essential for meiosis, thereby activating their expression (Fig. 2). Meisetz may form a chromatin-remodeling complex around each target gene [39, 40], and future studies including identification of proteins interacting with Meisetz may reveal detailed mechanisms of meiosis-specific gene activation by Meisetz. Genes activated by the functions of Meisetz may include those involved in pairing of homologous chromosomes and/or their recombination. Histone H3K4 methylation is widely found around regulatory regions of actively transcribed genes [33, 34], and it is likely that Meisetz also affects genes not specific for germ cells. However, a deficiency in the *Meisetz* gene causes specific abnormalities in meiotic prophase [38], suggesting that Meistez preferentially methylates functionally important genes for meiotic prophase.

Because Meisetz specifically trimethylates dimethylated histone H3K4 as a substrate, but cannot methylate non- and mono-methylated histone H3K4, it is most likely dimethylated before meiosis. This again suggests the importance of specific chromatin modifications and structures that must be established prior to the initiation of meiosis. Although we currently do not have any supporting evidence, Meisetz might also regulate meiotic chromosome structure. During early meiotic prophase, chromosomes generally undergo compaction of chromosome structure as described above, but a previous report has suggested that chromosome structure just before pairing is dynamically regulated and undergoes regional and transient decompaction [29]. Meisetz may be involved in this process, because histone H3K4 methylation could inhibit histone H3K9 methylation by Suv39h [41], and therefore it is most likely that Meisetz might induce regionally relaxed chromosome structures.

Although events during meiosis and at least a part of its mechanisms are largely conserved, evidence has indicated that there is sex-specific regulation of meiosis [42]. The fact that chromosomal abnormalities are more frequently observed in oocytes than in sperm may be consequent upon the sexually different regulation; male meiotic germ cells may be more strictly regulated than female ones with regard to checking errors during meiosis. Mutations in a number of genes involved in meiotic prophase show meiotic abnormalities only in the male, further supporting an idea that meiotic prophase is stringently controlled by more genes in the male than in the female [42]. Consistent with this idea, for molecules involved in epigenetic regulation in meiosis, mutations in Dnmt3a and Dnmt3L indeed cause male-specific meiotic failures [12–14], whereas Meisetz contributes equally to both male and female meiosis [38], and Suv39h1/h2 also seem to be involved in meiosis of both sexes [30]. Based on these facts, differences in epigenetic status between male and female may also be a critical factor in sex-dependent meiosis regulation. Identification of additional epigenetic regulators in meiotic germ cells in future stud-

ies may clarify sex-conserved and sex-specific aspects of epigenetic control of meiosis.

Conclusions

In mammals, RA may initially activate the molecular cascade required for early meiotic progression, and some epigenetic changes essential for meiosis are likely induced after this event. However, establishment of specific epigenetic status, such as DNA methylation by Dnmt3L/3a, as well as histone H3K4 dimethylation, is necessary before the induction by RA. During differentiation of primordial germ cells and spermatogonia, germ cells may set up all the epigenetic conditions [22] that cooperate with RA-induced cascades in establishing particular chromosome structures to initiate and promote meiosis. Genes required for meiosis may be directly or indirectly induced by RA, but RA-independent gene activation may also be necessary. Moreover, key transcriptional regulators such as Meisetz may initially define meiosis-specific gene expression.

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