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Resolving Molecular Events in the Regulation of Meiosis in Male and Female Germ Cells

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Abstract

In mammalian species, the process of meiosis, in which genes are randomly assorted between parental chromosomes during formation of egg and sperm cells, occurs prenatally in females but postnatally in males. To understand sex-specific differences in signaling mechanisms that underlie fertility, many studies have focused on identifying factors that control meiotic induction. Studies in mice using genetic knockout of the transcriptional regulator Polycomb repressive complex-1 (PRC1) and pharmacological inhibition of retinoic acid (RA) signaling suggest that PRC1 prevents female meiotic induction until release of PRC1 repression by increased RA signaling in the ovary. However, genetic studies with mice lacking RA synthesis in reproductive tissues indicate that RA is required for male but not female meiosis, suggesting that RA functions as a male-specific inducer of meiosis and that another factor releases PRC1 repression to initiate female meiosis. Correct resolution of the molecular events governing female and male meiosis is important for treating infertility and devising improved birth control strategies.

A distinctive feature of primordial germ cell (PGC) maturation is induction of meiosis, which in mammals occurs prenatally in females and postnatally in males (1–3). Three isoforms of retinaldehyde dehydrogenase (RALDH1, RALDH2, RALDH3) play essential roles in synthesis of retinoic acid (RA), a vitamin A metabolite essential for reproduction. Pharmacological studies suggested that RA produced by RALDH2 in the mesonephros may function as a diffusible sex-specific factor, inducing meiosis in the adjacent prenatal ovary, but not in the prenatal testis, which has the cytochrome P450 enzyme CYP26B1 that degrades RA (4, 5). However, genetic studies with *Raldh2*^{−/−} and *Raldh2*^{−/−};*Raldh3*^{−/−} mouse embryos demonstrated that loss of RA activity in the mesonephros and developing gonad has no effect on female meiosis (6). The double knockout was examined because *Raldh3* exhibits low expression in mesonephros; *Raldh1* is unlikely to compensate because RA activity was undetectable with a reporter in *Raldh2*^{−/−};*Raldh3*^{−/−} embryos in mesonephros and developing gonad (6).

Yokobayashi *et al.* (7) used gene knockout studies in mice to show that loss of Polycomb repressive complex-1 (PRC1) results in premature induction of female meiosis, indicating that PRC1 normally prevents female meiosis until this repression is released. Yokobayashi *et al.* (7) also reported that administration of an RALDH inhibitor prevents premature meiotic induction in PRC1-deficient ovary, and thus concluded that RA synthesis normally alleviates PRC1 repression to induce female meiosis. However, RALDH inhibitors have off-target effects, including inhibition of mitochondrial aldehyde dehydrogenase (8) and possibly other enzymes of this 20-member family that have functions other than RA synthesis. Thus, the pharmacological studies of Yokobayashi *et al.* (7) and others (4, 5) need

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to be carefully weighed against the genetic evidence demonstrating that elimination of RA synthesis does not prevent or delay female meiosis (6).

In mice, meiosis in the female gonad occurs at embryonic day 13.5 (E13.5). Yokobayashi *et al.* (7) propose that RA signaling increases in the ovary from E11.5 to E13.5 and that once it reaches a certain threshold, RA signaling overcomes PRC1 repression and allows meiotic induction. However, this conclusion conflicts with studies showing that RA activity monitored with the *RARE-lacZ* RA-reporter transgene does not increase in ovary between E12.0 and E13.5 (6). Indeed, *RARE-lacZ* expression is not detected in ovary during these stages, although it is easily detected in mesonephros and completely absent in the mesonephros of *Raldh2*^{-/-}; *Raldh3*^{-/-} mice (6). The sensitivity of *RARE-lacZ* had previously come into question (9), but recent studies have shown that *RARE-lacZ* is sensitive to 0.25 nM RA (10), lower than the 1 nM concentration shown to marginally induce meiosis in isolated PGCs and much lower than the 10 nM concentration needed for more complete meiotic induction in the dose-response curve (9).

Yokobayashi *et al.* (7) also reported that PRC1-deficient PGCs display low ectopic expression of *Raldh2*, but did not demonstrate that this leads to RA synthesis. Additionally, this is inconsistent with studies showing that PGCs are not the source of the factor that induces female meiosis; examination of isolated PGCs demonstrated that this factor is a diffusible extrinsic substance derived from mesonephric or ovarian somatic cells (3–6, 9). If a threshold level of RA is needed for female meiotic induction, as proposed by Yokobayashi *et al.* (7), then we would expect that loss of RALDH2 and 3 should reduce or delay this process even if RALDH1 plays a minor role, but the mouse genetic data do not support this model (6).

Stra8 encodes a cytoplasmic protein essential for both male and female meiosis, although its function in meiosis is unclear (4). Induction of *Stra8* is governed by sex-specific intrinsic and extrinsic factors and serves as a marker of meiotic induction. In the prenatal ovary, Doublesex and mab-3 related transcription factor-1 (DMRT1) plus WNT4 and WNT5A are essential for *Stra8* expression (1, 3), whereas in the prenatal testis, fibroblast growth factor 9 (FGF9) and CYP26B1 are essential to prevent *Stra8* expression (2, 4, 5) (Fig. 1). CYP26B1 loss-of-function studies in mice suggested that this enzyme degrades mesonephric-derived RA in the prenatal testis to prevent *Stra8* induction, which led to the assumption that mesonephric RA is required for *Stra8* induction in the prenatal ovary because it lacks CYP26B1 activity (4, 5); however, this assumption is not supported by RALDH genetic loss-of-function studies (6). By contrast, PGCs of postnatal testis require RA for *Stra8* induction as demonstrated by other RALDH genetic loss-of-function studies (11). Further, a role for RA in postnatal meiotic induction in males is supported by studies showing that PGCs in postnatal testis express *Raldh2* and locally generate RA (11), unlike PGCs in prenatal ovary and prenatal testis, which do not (4, 5). Therefore, the previously reported female *Stra8* response to pharmacological treatment with either RA (up-regulation) or RA receptor antagonists (down-regulation) (4, 5) may occur by invoking the RA-dependent male meiotic program (11); because genetic evidence indicates that female *Stra8* induction does not normally require RA (6), this would constitute an off-target effect.

By taking into account the genetic studies (1–3, 5–7, 11), we propose that RA is a male-specific factor controlling meiotic induction (Fig. 1). Further, as Yokobayashi *et al.* (7) reported that PRC1 represses *Stra8* not only in prenatal ovary but also in prenatal testis, we suggest that RA may release PRC1 repression of *Stra8* in testis postnatally. Several unanswered questions remain: What releases PRC1 repression during female meiosis prenatally? Does RA release PRC1 repression during male meiosis postnatally, and does this occur directly on the *Stra8* gene at the transcriptional level? Are some defects in human

fertility due to abnormal RA signaling or aberrant PRC1 function? What are the implications for clinical strategies to alter fertility—either infertility or birth control? On the basis of our sex-specific model of RA action during meiosis, therapies designed to increase RA signaling might treat infertility only in males, and strategies for birth control that decrease RA signaling might also be male specific. With genetic studies guiding the way, further progress in understanding the molecular basis of fertility is on the horizon.

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Fig. 1. Proposed model for sex-specific control of meiotic induction based on genetic findings RA generated in the mesonephros acts locally to control differentiation of Müllerian (md) and Wolffian (wd) ducts (12), but diffusion of mesonephric RA to prenatal ovary or prenatal testis is insufficient to induce meiosis in prenatal PGCs that lack enzymes to locally generate RA (6). By contrast, RA is generated locally in PGCs of postnatal testis and is required to induce *Stra8* for male meiotic induction (11); RA produced in postnatal male PGCs may directly or indirectly release PRC1 repression to induce *Stra8*. M, mesonephros; Ov, ovary; Tes, testis.