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Minireview

Initiating Meiosis: The Case for Retinoic Acid[1]

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ABSTRACT

The requirement for vitamin A in reproduction and development was first determined from studies of nutritional deficiencies. Subsequent research has shown that embryonic development and both male and female reproduction are modulated by retinoic acid (RA), the active form of vitamin A. Because RA is active in multiple developmental systems, its synthesis, transport, and degradation are tightly regulated in different tissues. A growing body of evidence implicates RA as a requirement for the initiation of meiosis in both male and female mammals, resulting in a mechanistic model involving the interplay of RA, RA synthesis enzymes, RA receptors, and degradative cytochrome P450 enzymes in this system. Recently, that model has been challenged, prompting a review of the established paradigm. While it remains possible that additional molecules may be involved in regulating entry into meiosis, the weight of evidence supporting a key role for RA is incontrovertible.

germ cells, meiosis, ovary, retinoic acid, retinoids, testis

INTRODUCTION

Nearly a century ago, E.V. McCollum established that a fat-soluble micronutrient (''factor A'') was necessary to sustain life and prevent blindness in cows and rats [1]. Since that time, vitamin A has been found to have essential roles not only in vision but also in skin, bone, immune system, and reproductive health, as well as in many aspects of embryonic development. Because of the essential nature of this vitamin, all steps in its metabolism—including the absorption of precursors, storage of retinol esters, oxidation of these esters to the primary active metabolite retinoic acid (RA), and degradation of RA to inactive metabolites—are subject to tight biological controls and are protected by genetic redundancy. Retinoic acid commonly acts in a paracrine manner, with one cell type controlling the storage and oxidation of retinol (ROL), while a second cell type serves as the target for the action of RA [2]. In the signaling cells, conversion of ROL to RA requires two sequential oxidative steps, catalyzed by retinol or alcohol dehydrogenases and by retinaldehyde dehydrogenases (RALDHs), respectively. In the responding cells, RA serves as a ligand for two families of nuclear receptors, the RA receptors (RARs) and the retinoid X receptors (RXRs). The RA:RAR/RXR complex binds to RA response elements (RAREs) in target genes, recruiting corepressors or coactivators and thereby bringing about transcriptional changes. The level of RA present in a given tissue is finely tuned by the balance between its synthesis by RALDHs and its oxidative degradation by the following cytochrome P450 enzymes: cytochrome P450, family 26, subfamily a, polypeptide 1 (CYP26A1); cytochrome P450, family 26, subfamily b, polypeptide 1 (CYP26B1); and cytochrome P450, family 26, subfamily c, polypeptide 1 (CYP26C1) [2].

In mammals, the timing of germ cell entry into meiosis differs between the sexes. The first histological evidence that germ cells in the fetal mouse ovary have embarked upon meiosis I is seen at about 13.5 days postcoitum (dpc) [3]. The formation of meiotic chromosomal figures is preceded by expression of the premeiotic marker gene stimulated by RA gene 8 (*Stra8*) at about 12.5 dpc [4–6]. On the other hand, testicular germ cells do not enter meiosis during fetal life; in mice, they first express *Stra8* shortly after birth, and expression peaks at about 10 days postpartum (dpp), coincident with the onset of meiosis I [7]. The upregulation of *Stra8* is critical for both oogenesis and spermatogenesis: in the absence of *Stra8* function, the progression of germ cells through meiosis in both the fetal ovary and the postnatal testis is blocked [4, 8, 9].

In recent years, independent investigations from numerous laboratories have yielded a large body of evidence demonstrating that RA triggers the onset of meiosis in both male and female mammals, including mice and rats (references detailed herein), as well as in other vertebrates, including chickens and amphibians [10, 11]. In humans, the role of RA in meiosis has been demonstrated only in the ovary [12, 13]. In the mouse, the organism most thoroughly studied thus far, the evidence suggests that RA, produced in the adjacent mesonephros, acts in the fetal ovary to trigger the onset of meiosis in germ cells. In the fetal testis, endogenous RA is evidently cleared by CYP26B1, ensuring that the onset of meiosis in male germ cells is delayed until after birth [5, 14, 15]. In the early postnatal and adult testis, RA is also required to upregulate *Stra8* and sustain meiosis [14, 16]. As a result of these studies, the paradigm has become that the balance between RA synthesis and degradation in the developing reproductive system is required for appropriate control of the induction of *Stra8* expression and hence the timing of meiotic initiation (Fig. 1).

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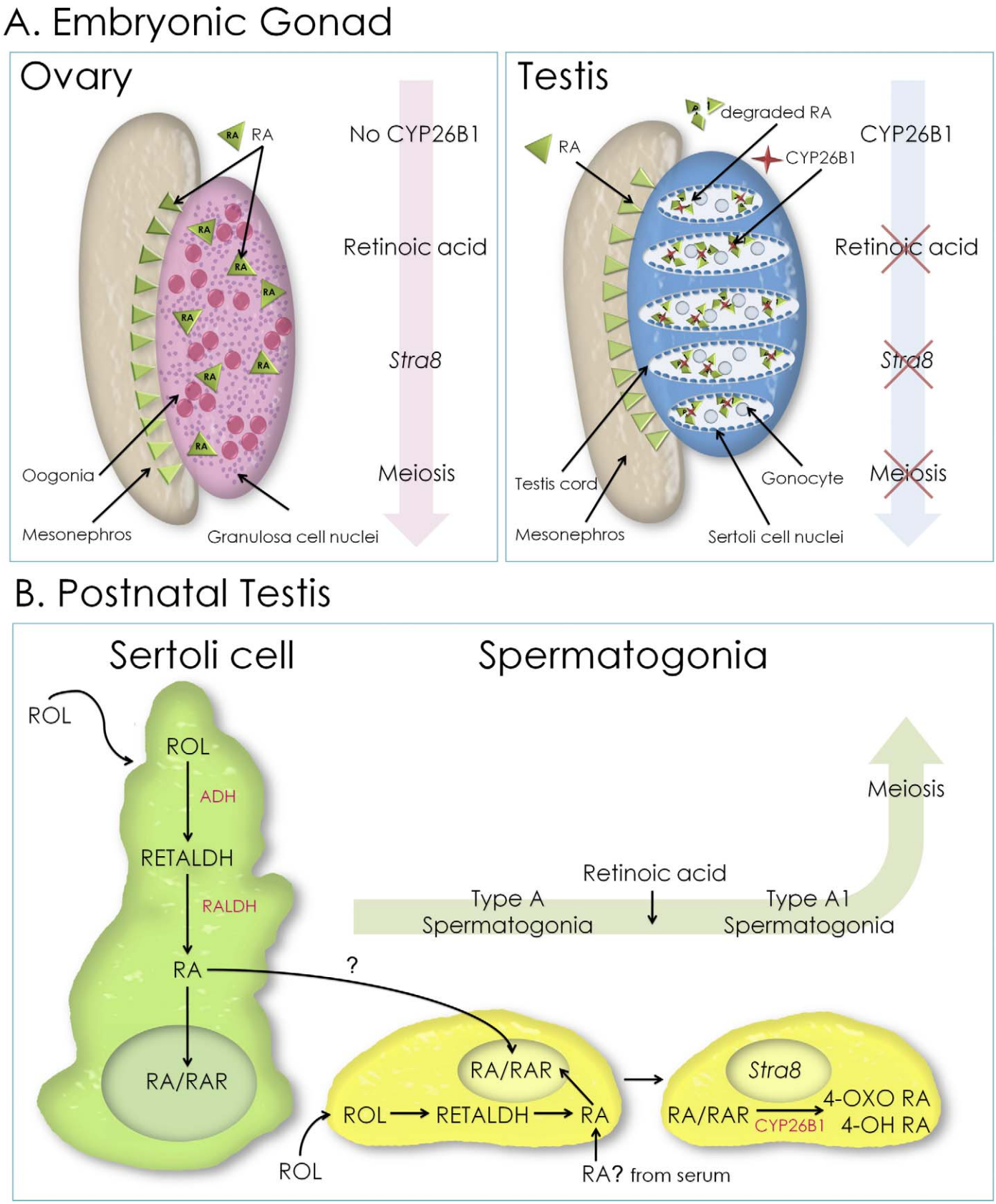

FIG. 1. The current paradigm for RA driving the onset of meiosis in males and females. **A**) In the embryonic ovary, RA (green triangles) generated in the mesonephros drives the expression of *Stra8* and the onset of meiosis in the oogonia (pink). In the male embryo, RA is still produced in the mesonephros; however, the action of CYP26B1 (red stars), an RA-metabolizing enzyme that is made by Sertoli cells and testicular interstitial cells, acts to degrade this RA, thereby preventing *Stra8* expression and the onset of meiosis. **B**) In the postnatal testis, there are several possible ways in which RA might be generated and delivered to spermatogonia. Retinol (ROL) may be internalized by either Sertoli cells or spermatogonia and converted first to retinaldehyde (RETALDH) and then to RA via a two-step process catalyzed by the alcohol dehydrogenases (ADH) and the aldehyde/retinaldehyde dehydrogenases

Data from a recent study [17] have challenged this view. In that study, meiosis was examined in fetal ovaries null for either aldehyde dehydrogenase family 1, subfamily A2 (*Aldh1a2*) (also known as RALDH 2 [*Raldh2*]) alone or in combination with aldehyde dehydrogenase family 1, subfamily A3 (*Aldh1a3*) (also known as RALDH 3 [*Raldh3*]). Because *Stra8* was still expressed and meiosis markers synaptonemal complex protein 3 (*Scp3*) transcript and H2A histone family, member X (γH2AFX) protein were still detectable, the authors concluded that meiosis had occurred normally, despite the absence of two major RA-synthesizing enzymes. These authors were unable to detect RA in the developing ovary using a transgenic RARE-*LacZ* reporter mouse line, nor were they able to demonstrate strong binding of RARs to *Stra8* regulatory sequences. Curiously, they did find that the inhibition of CYP26B1 action in fetal testes led to the ectopic upregulation of *Stra8* and that this occurred only when the mesonephros was present. The authors concluded 1) that RA is not present in the developing mouse ovary and is therefore not required for meiosis to begin in XX fetal germ cells and 2) that the role of CYP26B1 in the developing fetal testis is to degrade a nonretinoid meiosis-inducing factor that diffuses in from the mesonephros.

Clearly, these data do not fit comfortably with the established model for RA-driven control of germ cell entry into meiosis (Fig. 1) and force a reconsideration of available evidence. In this review, we will evaluate the data supporting the established role for RA as a molecular regulator of germ cell entry into meiosis and the new data underpinning the challenge proposed by Kumar et al. [17] in the hope of reconciling the apparently conflicting observations.

THE CASE FOR RA

Evidence for the involvement of RA in germ cell meiosis derives from observations and experiments in a number of species spanning the last decade. These investigations have led to seven major conclusions supporting the model shown in Figure 1, as discussed in the following sections.

Initiation of Meiosis I in Both Spermatogenesis and Oogenesis Is Blocked by Dietary Retinoid Deficiency

The effects of vitamin A deficiency or retinoid supplementation on spermatogenesis in rats and mice are well documented. Long-term vitamin A deprivation results in spermatogenic arrest at the spermatogonial A to A1 transition (undifferentiated to differentiated spermatogonia) or at the preleptotene spermatocyte stage in rats, and primarily at the A to A1 transition in mice [18–23]. When ROL is provided to vitamin A-deficient (VAD) rodents, meiosis is reinitiated promptly and synchronously [18, 20, 22]. Large doses of RA can also induce resumption of meiosis in this system, suggesting that RA, and not its precursor ROL, is the active factor [23]. Recent findings have extended our understanding of this phenomenon: ROL injection into VAD mice dramatically induced *Stra8* expression over a 24-h period, indicating that ROL rescue in VAD rodents involves induction of expression of this critical premeiotic gene [14]. Retinoic acid is also required for initiation of meiosis during the first wave of mouse spermatogenesis. In postnatal male mice null for lecithin:retinol acyltransferase (*Lrat*), which are particularly susceptible to becoming VAD, dietary depletion of vitamin A resulted in loss of *Stra8* expression, the accumulation of undifferentiated spermatogonia, and meiotic failure [24].

Li and Clagett-Dame [25] used VAD rats to study germ cells in the developing ovary. They observed that the majority of germ cells in ovaries from severely VAD embryos failed to induce *Stra8*, failed to enter meiosis, and remained undifferentiated. In addition, in a group of animals that was moderately deficient in RA, only about 30% of the oogonia entered meiosis compared with 75% in the controls. These in vivo experiments demonstrated a dose-dependent requirement for RA to initiate meiosis in the fetal gonad at the exact developmental point that the established paradigm predicts. Moreover, they demonstrated that RA is necessary, not just sufficient, to initiate meiosis.

RA Induces Expression of the Meiotic Gatekeeper Gene Stra8

Gene knockout studies have firmly established that expression of the premeiotic gene *Stra8* is indispensable for entry into meiosis in the fetal ovary [4] and for the switch from mitosis to meiosis in the adult testis [8, 9]. When *Stra8* was knocked out in mice on a pure C57BL/6 background, preleptotene germ cells did not enter meiosis [8]; however, if the knockout was done on a mixed background, some of the cells completed meiotic replication and entered meiosis but failed to complete prophase [9]. *Stra8* was identified as an RA-responsive gene in P19 embryonal carcinoma cells: RA treatment leads to upregulation of *Stra8* within 2 h [26, 27]. Indeed, the gene designation *Stra8* is an acronym for ''stimulated by RA gene 8.'' Treatment of mouse fetal gonadal tissue or adult testis tissue with RA ex vivo also leads to upregulation of *Stra8* [5, 7, 14, 28–30].

Meiosis Is Triggered by Exogenous Retinoids

An early indication that retinoids might be involved in meiotic initiation came from the observation that treatment of 14.5-dpc rat ovary explants with RA or an RARα-specific agonist accelerated entry into meiosis [31]. More recently, it has been demonstrated that exogenous RA can stimulate germ cells in the embryonic testis to enter meiosis, as determined by expression of bona fide meiotic markers *Scp3*, DMC1 dosage suppressor of mck1 homolog, meiosis-specific homologous recombination (yeast) (*Dmc1*), and *γH2afx* and by morphological analysis [5, 14, 15, 29]. Exogenous RA cannot rescue the loss of meiosis in *Stra8*-null fetal ovaries [14], implying that the role of RA is to induce *Stra8* that then triggers chromatin condensation characteristic of meiotic prophase.

Several other studies had either found no effect of RA on meiosis or overlooked any possible effect. When Best and colleagues [32] cultured 11.5-dpc urogenital ridges with exogenous RA, they found that *Stra8* was expressed but that only 1% of XY germ cells had entered meiosis. It remains unclear why the observations in that study differ from those by other groups. Other investigators looking at the effects of RA on rat testis development did not find that RA triggered meiosis, but they did not specifically look for such an effect

(RALDH). Retinoic acid may also be delivered directly from the serum. Once inside the cell, RA can interact with RARs and stimulate transcription of a number of genes, including *Stra8*. The expression of *Stra8* in spermatogonia, stimulated by RA, is coincident with the transition from undifferentiated type A spermatogonia to differentiated type A1 spermatogonia. Excess RA can be metabolized by the enzyme CYP26B1 into 4-oxo (OXO) and 4-hydroxy (OH) forms. These retinoid forms are then secreted from the cell.

[33–35]. Those studies found that RA treatment for 3 days induced dose-dependent apoptosis of the germ cells: such an observation is not at odds with the possibility that XY germ cells in the testis entered meiosis and were subsequently removed by apoptosis, a well-known phenomenon [15, 36–38].

Meiosis Is Triggered by Endogenous Retinoids

The effects of increasing endogenous RA levels have been tested in a number of studies in which the degradation of RA in mouse fetal testes has been inhibited using strategies aimed at blocking CYP26B1 activity. When CYP26B1 is inhibited pharmacologically using the potent but nonspecific cytochrome P450 inhibitor ketoconazole, resident germ cells of the fetal testis upregulate *Stra8*, *Scp3*, and *Dmc1* and take on morphological features of meiotic germ cells [5, 14]. Although ketoconazole is capable of inhibiting P450 enzymes other than CYP26B1, it is clear that the action of ketoconazole in the induction of meiosis in this system relies on signaling through RARs: when ketoconazole and the RAR panantagonist BMS-204493 were used in tandem in ex vivo organ culture, meiosis was not induced [14]. The same conclusion was drawn when a more specific inhibitor of CYP26 enzymatic activity, R115866, was tested in parallel with the RAR panantagonist [14]. In the prepubertal testis, inhibition of CYP26 with R115866 has also been used to demonstrate an increase in differentiated spermatogonia (*Stra8* and kit oncogene (*Kit*) positive) and a decrease in undifferentiated spermatogonia (POU domain, class 5, transcription factor 1 [*Pou5f1*] positive) [16]. Taken together, these studies strongly imply that induction of *Stra8* and entry into meiosis require RAR signaling and that a CYP26 enzyme opposes this action.

More specific evidence for the importance of CYP26B1 comes from studies of *Cyp26b1*-knockout mice. These mice die immediately after birth of respiratory distress [15, 39]. In *Cyp26b1*-null fetal testes, germ cells strongly upregulated *Stra8* and *Scp3*, and by 16.5 dpc some germ cells had progressed as far as the pachytene stage of meiosis I [5, 15]. Importantly, a threefold increased level of RA was detected, confirming that removal of *Cyp26b1* had, as expected, allowed endogenous RA to build up in the tissue [15]. To demonstrate that the meiotic induction observed in the *Cyp26b1*-knockout mouse was the result of the presence of ectopic RA accumulation (rather than some other effect of the loss of *Cyp26b1*), wild-type fetal testes were treated with an RAR agonist (AM580) that is resistant to the actions of CYP26B1 [15]. As expected, XY germ cells in such testes promptly expressed *Stra8* and entered meiosis. Even later in development, when XY germ cells in an embryonic testis have already entered mitotic arrest, they can be triggered to upregulate *Stra8* and enter meiosis by ectopic exposure to endogenous RA. When *Cyp26b1* was deleted specifically in Sertoli cells from about 15.5 dpc, *Stra8* was induced at about 16.5 dpc, and XY germ cells entered meiosis [25].

Meiosis Is Inhibited by RAR Antagonists and Stimulated by RAR Agonists

If RA directly induces *Stra8* expression and thereby triggers entry into meiosis, it is necessary that RARs are expressed by germ cells. This has been demonstrated for both fetal ovarian germ cells [5, 40, 41] and prepubertal/adult testis germ cells [40, 42, 43]. Furthermore, studies have shown that both *Stra8* induction and the entry into meiosis can be inhibited in the embryonic ovary by the action of RAR antagonists. In two separate studies [5, 14], the RAR panantagonists BMS-204493 and AGN193109 were shown to inhibit *Stra8*, *Scp3*, and *Dmc1* expression in 11.5-dpc mouse ovaries in culture. Conversely, *Stra8* was induced in embryonic testes cultured with agonists specific for each of the three RARs, demonstrating that any one of the three RAR isotypes is capable of activating *Stra8* expression in the developing gonad [14].

Meiosis Is Blocked by ALDH Inhibitors

Bis-(dichloroacetyl)-diamines (BDADs) are compounds that inhibit spermatogenesis. Recent investigations have demonstrated that the BDAD WIN 18,446 acts by inhibiting the biosynthesis of RA from ROL and, consequently, blocks the expression of *Stra8* in embryonic gonads, neonatal and adult testes, and isolated 2-dpp germ cells [44]. Expression of *Stra8* can be rescued by exogenous RA but not by ROL. In a complementary study [45], WIN 18,446 was shown to inhibit RALDH2, the major enzyme of RA biosynthesis, in vitro and in vivo, in rabbit testes. After treatment, intratesticular RA levels were decreased, spermatogenesis was impaired, and rabbits became infertile. The RALDH inhibitor citral also blocks meiosis in mouse and human fetal tissues [5, 13, 46].

RA Is Present at the Right Place and Time to Drive Meiosis

Given that germ cells enter meiosis in the fetal ovary but not in the fetal testis, one would expect to be able to detect the presence of RA in the ovary but not in the testis. Using the RARE-*hsplacZ* indicator mouse [47], RA was indeed detected in the developing mouse ovary [5]. Although β-galactosidase reporter staining in the developing ovaries was weak, no staining at all was observed in stage-matched fetal testes, in which *Cyp26b1* is strongly expressed. Presumably, low levels of endogenous RA are sufficient to drive meiosis in the context of the fetal gonad.

The presence of RA detected by RARE-*lacZ* reporter mice does not reveal where the RA is actually made but rather its sites of action. To resolve this issue, gonads and mesonephroi were cultured on a lawn of F9 RARE reporter cells that respond to tissues that actively synthesize RA but not those that merely harbor RA. This assay was used to show that the mesonephros, but neither the ovary nor the testis, synthesizes RA [5]. This result was in agreement with the finding of strong *Aldh1a2* expression in the mesonephros [5]. Thus, it was hypothesized that RA is produced in the mesonephric duct and tubules and that it diffuses into the adjacent gonad, triggering *Stra8* expression and meiosis in resident germ cells. A recent study that seemingly challenges this hypothesis was published by Livera and colleagues [31]. They found that, when the mesonephros was removed from 11.5-dpc ovaries and those ovaries were then cultured, germ cells still enter meiosis. As the authors point out, however, this does not prove that RA is not involved in meiotic initiation, because it cannot be ruled out that some RA is produced within the fetal ovary itself [30]. In other words, this study challenges the source of RA but not the model that RA is the driver of entry into meiosis.

In recent investigations, Snyder et al. [16] used the same RARE-*lacZ* reporter mice to examine the role of RA in the initiation of meiosis during the first round of spermatogenesis in neonatal mice. They found that all germ cells expressing β-galactosidase, indicating response to endogenous RA, also expressed *Stra8*. When the neonates received injections of exogenous RA, the number of germ cells positive for RA activity and for *Stra8* increased. Positive cells were predominantly differentiated premeiotic germ cells.

In summary, the combined weight of published data presents a strong case for the action of RA in triggering germ cell entry into meiosis. In particular, the block in meiotic progression in males and females as a result of dietary vitamin A insufficiency provides unequivocal evidence for the requirement for RA to complete this crucial biological process. Nonetheless, the recent findings by Kumar et al. [17] potentially cloud the issue.

MEIOSIS IN THE ABSENCE OF RA SIGNALING?

Somewhat surprisingly, given the case already outlined, Kumar et al. [17] found that germ cells in the fetal ovary could enter meiosis in *Aldh1a2*-knockout or *Aldh1a2*/*Aldh1a3*-double-knockout mice. They were unable to detect RA in the developing ovary using a transgenic RARE-*LacZ* reporter mouse line, nor were they able to demonstrate strong binding of RARs to *Stra8* regulatory sequences. However, consistent with other studies, inhibition of CYP26B1 in fetal testes led to the ectopic upregulation of *Stra8*, albeit only when the mesonephros was present. Based on their observations, Kumar et al. do not dispute the central role of *Stra8* in the onset of meiosis, nor do they disagree that CYP26B1 is key to the avoidance of meiosis in the fetal testis. They postulate that a substance, not a retinoid, diffuses into the gonad from the mesonephros and is degraded by CYP26B1. The suggestion that entry into meiosis is completely independent of RA is controversial and has attracted a great deal of interest in the field. Just how sound is the evidence that RA is not involved?

We suggest that alternative interpretations are possible for several of the observations by Kumar et al. [17]. First, Kumar et al. did not factor aldehyde dehydrogenase family 1, subfamily A1 (ALDH1A1) (also known as retinal dehydrogenase 1 [RALDH1]) into their system. *Aldh1a1* is highly expressed in the fetal testis, and although expression is much lower in the fetal murine ovary, it is detectable via both quantitative RT-PCR and microarray analysis [48]. In the human embryonic ovary, all three RALDHs have been shown to be expressed, and it is postulated that ALDH1A1 may be the one that actually drives meiosis in that system [12, 13].

But if ALDH1A1 is present at any significant level in the embryonic ovary, why was no RA signaling activity detected in the study by Kumar et al. [17]? The RARE-*hsplacZ* reporter mouse line is notoriously prone to losing responder activity [49], and while it is clear that RA levels in the *Aldh1a2*-knockout and *Aldh1a2*/*3*-knockout mice are reduced relative to wild type, no quantitative evidence has been presented that RA is completely absent from these gonads. A negative result showing failure to find something is not the same as proving that something is not there. We suggest that, given that *Aldh1a1* mRNA can be detected in the embryonic ovary, the action of this enzyme may generate enough RA to explain the results seen in the mutant mice in the study by Kumar et al.

Second, the mouse model used by Kumar et al. [17] relies on the supply of exogenous RA to pregnant mothers for a period around gastrulation to overcome lethality associated with RA deprivation during development. The necessity of deliberately adding a molecule when one is attempting to assay the consequences of the absence of that molecule complicates the experimental system, and the presence of residual RA at low levels, or the delayed effects on germ cells of early exposure to RA, cannot be completely discounted.

Third, Kumar et al. [17] performed chromatin immunoprecipitation (ChIP) on 13.5-dpc tissue from mesonephros and ovary to assay the ability of RARs to bind to the *Stra8* promoter. They showed that the binding of all three RARs to the RARE present upstream of *Stra8* was significantly less than the binding of these receptors to the RARE present in the RAR beta (*Rarb*) gene. However, the weakness of the ChIP signal could be due to the low representation of germ cells in the samples assayed, which comprised all mesonephric cells and somatic cells of the ovary in addition to the target germ cell population. We speculate that RARs might be shown to bind more convincingly if the starting material had consisted of purified germ cells; other studies [14, 28] clearly indicate that only germ cells, at a particular time during their development, are able to respond to RA by upregulating *Stra8*. We do not concur that the data by Kumar et al. convincingly establish that *Stra8* is not a normal target of RA, but it is clear that further studies will be required to address this point.

Fourth, Kumar et al. [17] postulate that a meiosis-inducing substance diffuses into the gonad from the mesonephros and is degraded by CYP26B1. Given that CYP26B1 is not known to degrade nonretinoid compounds, and that these properties match exactly those established for RA, we suggest that the simplest explanation for the findings by Kumar et al. is that their observations are attributable to RA.

RECONCILING ESTABLISHED AND NEW FINDINGS

Skepticism regarding an established model is not a bad thing. Although it is possible to question some of the experimental findings that make up the body of evidence in support of the role of RA in driving entry into meiosis, we are unable to identify any issues that discount a role for RA completely. For example, it has been suggested that the induction of *Stra8* and the initiation of meiosis by exogenous RA reported in various publications result from the ''supra-physiological'' concentrations used [17]. Most of the ex vivo organ culture studies have used concentrations of RA ranging from 0.7 to 1 μM RA. However, given that whole tissues were treated in these experiments, it is likely that target cells are exposed to lower concentrations of RA than those initially present in the media. Furthermore, at least three studies [7, 50, 51] have demonstrated that RA acts directly on isolated germ cells (i.e., not via Sertoli or any other gonadal somatic cell type) to trigger *Stra8* expression. When individual target cells (i.e., purified germ cells) are studied in culture, far lower RA concentrations are required to trigger meiosis [5]. In these experiments, as little as 1 nM RA is sufficient to induce *Stra8* expression; the effect is dose dependent, and the levels of RA required are in keeping with concentrations estimated to be present in embryonic tissues (10–40 nM in most target tissues and 11 nM in the adult murine testis) [47, 52].

It has also been suggested that the actual physiological role of RAR antagonists may be different from their reported functions because these compounds may exert nonspecific effects on other receptors [17], based on a known case involving the RAR antagonist Ro 41–5253 [53]. This is a fair point, although the two pan-RAR antagonists that have been shown to inhibit meiosis are BMS-204493 and AGN193109, not Ro 41–5253 [5, 14]. The same authors argue that commonly used RALDH inhibitors, including disulfiram compounds such as WIN 18,446 and citral, can also inhibit aldehyde dehydrogenases that have no relevance to retinoid metabolism. While this is possible, the reversal of the WIN 18,446 inhibition of *Stra8* induction by RA but not ROL strongly indicates that the significant inhibitory action of WIN 18,446 on meiosis is on the RALDHs [44].

The identification of intrinsic factors and modifiers affecting meiosis entry is currently limited. Deleted in azoospermia-like (DAZL), an RNA-binding protein expressed in postmigratory

germ cells, is one intrinsic factor that must be expressed if germ cells are to respond to RA by initiating meiosis [28]. Similarly, doublesex and mab-3 related transcription factor 1 (DMRT1) expressed in spermatogonia appears to restrict RA responsiveness and repress the transcription of *Stra8*, thereby opposing meiotic entry [54], while it seems to have the opposite role in the fetal ovary [55]. Clearly, the field has only just begun to understand the details of how the intrinsic and extrinsic factors that regulate meiosis are produced in or delivered to the embryonic ovary and postnatal testis and how signaling pathways are activated and gene expression and protein changes occur in germ cells in response to these factors.

The control of the onset of meiosis is obviously crucial for successful reproduction. The current paradigm shown in Figure 1 has been challenged by the findings by Kumar et al. [17], but there remains strong evidence for the action of RA in the induction of *Stra8* and the onset of meiosis in both sexes. While there are ample experimental data supporting this paradigm, the entire story is likely incomplete. Retinoic acid is clearly necessary given the weight of evidence that deficiency of vitamin A or inhibition of RA action precludes normal meiotic behavior in both male and female germ cells. However, it may not be the only secreted inducer of meiosis. Future studies will determine if other inducers exist and how they may interact with RA action.

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