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# Meiosis and retinoic acid in the mouse fetal gonads: an unforeseen twist.

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## Abstract

In mammals, differentiation of germ cells is crucial for sexual reproduction, involving complex signaling pathways and environmental cues defined by the somatic cells of the gonads. This review examines the long-standing model positing that all-*trans* retinoic acid (ATRA) acts as a meiosis-inducing substance (MIS) in the fetal ovary by inducing expression of STRA8 in female germ cells, while CYP26B1 serves as a meiosis-preventing substance (MPS) in the fetal testis by degrading ATRA and preventing STRA8 expression in the male germ cells until postnatal development. Recent genetic studies in the mouse challenge this paradigm, revealing that meiosis initiation in female germ cells can occur independently of ATRA signaling, with key roles played by other intrinsic factors like DAZL and DMRT1, and extrinsic signals such as BMPs and vitamin C. Thus, ATRA can no longer be considered as ‘*the*’ long-searched MIS. Furthermore, evidence indicates that CYP26B1 does not prevent meiosis by degrading ATRA in the fetal testis, but acts by degrading an unidentified MIS or synthesizing an equally unknown MPS. By emphasizing the necessity of genetic loss-of-function approaches to accurately delineate the roles of signaling molecules such ATRA *in vivo*, this chapter calls for a reevaluation of the mechanisms instructing and preventing meiosis initiation in the fetal ovary and testis, respectively. It highlights the need for further research into the molecular identities of the signals involved in these processes.

## Abbreviations

AGN193109	inverse agonistic ligand for all RAR isotypes, from Allergan, Inc
ATRA	all- <i>trans</i> retinoic acid
BMP	bone morphogenetic protein
BMS-194753	agonistic ligand selective for RARA, from Bristol-Myers Squibb pharmaceuticals
BMS-213309	agonistic ligand selective for RARB, from Bristol-Myers Squibb pharmaceuticals
BMS-189394	agonistic ligand selective for RARG, from Bristol-Myers Squibb pharmaceuticals
BMS-204493	inverse agonistic ligand for all RAR isotypes, from Bristol-Myers Squibb pharmaceuticals
CYP	cytochrome P450 enzymes
CYP26	cytochrome P450 family 26 enzymes
E	embryonic day
MIS	meiosis-inducing substance

MPS	meiosis-preventing substance
PGC	primordial germ cells
RAR	retinoic acid receptor
RARE	retinoic acid-responsive element
VAD	vitamin A deficient
WIN-18,446	bis(dichloroacetyl)diamine, inhibitor of retinaldehyde dehydrogenase

## 1. Introduction

In mammals, the hallmark of sex reproduction is the joining of a female and a male haploid germ cells that reconstitute a diploid embryo at the basis of the next generation. The germ cells of both sexes arise from a common cell-type, called the primordial germ cells (PGC), that either follow the female or the male pathway of differentiation named oogenesis or spermatogenesis, respectively. The PGC are formed as a small cohort of proximal epiblast cells that move into the extraembryonic tissues at the base of the allantois (Ginsburg, Snow & McLaren, 1990; Lawson & Hage, 1994). They proliferate and migrate along the hindgut of the embryo to reach the genital ridges. These structures arise from the intermediate mesoderm and are initially bipotential. Under the influence of the WNT4/RSP01/CTNNB1 signaling pathways, the female genital ridges develop as ovaries (Chassot, Gillot & Chaboissier, 2014), while under the influence of the SRY/SOX9/FGF9 signaling pathways, the male genital ridges develop as testes (Kobayashi, Chang, Chaboissier, Schedl & Behringer, 2005). In the mouse, this occurs at around embryonic day (E) 10.5 to E11.5 (McLaren, 1988). PGC continue to proliferate for a few days, so that about 25 000 germ cells reside in the fetal mouse gonads at E13.5 (Tam & Snow, 1981). It is only when sex determination of the gonadal soma has occurred that germ cells commit to the female, or the male differentiation pathway (McLaren & Southee, 1997). Indeed, the first morphological sign of sex-specific germ cell differentiation is seen at around E13.5, when female germ cells stop proliferating and initiate meiosis in the fetal ovary (McLaren & Southee, 1997; McLaren, 2003). This is done thanks to the expression of STRA8 and MEIOSIN proteins (reviewed in Ishiguro, 2023). The oocytes then proceed through the leptotene, zygotene and pachytene stages of the first meiotic prophase division. They arrest at diplotene stage and remain in this state until just before ovulation postnatally, at which time they complete the first meiotic division, begin the second one and arrest again. Meiosis is completed only after fertilization (Colas & Guerrier, 1995). In contrast, male germ cells do not initiate meiosis in the fetal testis. They arrest in the G0 or G1 phase of the mitotic cycle between E13.5 and E14.5, and enter a period of quiescence (Hilscher et al., 1974). After birth, the male germ cells resume mitosis and some differentiate as spermatogonia stem cells. It is only at puberty that some daughter spermatogonia initiate meiosis (Clermont, 1972).

For a long time, it was unknown whether germ cells were primed to enter meiosis by default, or if they were instructed by signals coming from the environment generated by the somatic cells surrounding them (Donovan, Stott, Cairns, Heasman & Wylie, 1986). Studies with chimeric mice have shown that germ cells, whether female or male, enter meiosis when they are embedded into a fetal ovary, but do not if in a fetal testis. Thus, the initial sex-specific differentiation of a germ cell is determined by its gonadic environment, but not by its chromosomal content (Evans, Ford & Lyon, 1977; McLaren, 1983; McLaren, 1995). Three possible scenarios could account for this observation: (i) somatic feminizing factors in the ovary promote meiotic initiation, while quiescence is established by default in the testis; (ii) somatic masculinizing factors in the testis promote quiescence, while meiotic entry occurs spontaneously and cell-autonomously in the ovary; (iii) both feminizing and masculinizing factors instruct meiosis in ovaries and quiescence in testes, respectively (Bowles & Koopman, 2007; Kocer, Reichmann, Best & Adams, 2009). Earlier studies involving co-culture of gonads have suggested that a diffusible “meiosis-inducing substance” (MIS) originating from the cranial part of the mesonephros (i.e., *rete ovary/rete testis*) triggers male germ cells to enter meiosis (Byskov, 1974; Byskov & Saxen, 1976). They also showed that a “meiosis-preventing substance” (MPS) diffusing from the fetal testis itself blocks meiosis in the female germ cells (Byskov, 1978). Accordingly, the prevailing model was that a MIS is present both in the ovary and the fetal testis, while an MPS is active only in the fetal testis (Bowles & Koopman, 2007; Kocer, Reichmann, Best & Adams, 2009). However, the nature of both the MIS and the MPS remained unknown for years.

## 2. ATRA and CYP26B1 as meiotic inducing and preventing substances

The discovery that Stimulated by retinoic acid, clone number 8 (*Stra8*) is specifically expressed in female germ cells at E12.5, just prior to entering meiosis (Menke, Koubova & Page, 2003), while *Cyp26b1* is restricted to testis somatic cells at E13.5, just prior to quiescence of male germ cells (Menke & Page, 2002) has given a new impetus. Actually, because (i) *Stra8* expression is induced by pharmacological levels of ATRA in cultured cells (Oulad-Abdelghani et al., 1996), (ii) CYP26B1 is identified as an ATRA degrading enzyme (White et al., 2000), (iii) the mRNA coding the ATRA-producing enzyme ALDH1A2 is expressed in the mesonephros (Neiderreither, McCaffery, Dräger, Chambon & Dollé, 1997), and (iv) STRA8 is essential to trigger meiotic entry (Baltus et al., 2006; Mark et al., 2008), an elegant model was devised, whereby ATRA plays the role of MIS and CYP26B1 plays the role of MPS (Koubova et al., 2006; Bowles et al., 2006). According to this new paradigm, ATRA is synthesized by the mesonephros in both sexes and it diffuses into the ovary and the testis through the rete ovary/rete testis. Within the ovary, ATRA drives the expression of *Stra8*, which triggers meiotic initiation. This fits perfectly well to the characteristics expected for a MIS. Within the testis, Sertoli cells encasing germ cells express CYP26B1, and form thereby a catabolic barrier that degrades ATRA coming from mesonephros. As a result, germ cells in the fetal testis are not exposed to the MIS, and they become quiescent. In this scenario the MPS is not a secreted molecule but rather a cytosolic enzyme (Bowles & Koopman, 2007).

The model according which ATRA and CYP26B1 are the long-sought MIS and MPS, respectively, was set up in the mouse, deduced essentially from expression patterns, and functionally tested by using exogenous retinoids added to organotypic culture experiments at levels much higher than endogenous ATRA, with the exception of CYP26B1, the role of which was studied in part by analyzing *Cyp26b1*-null fetal testes. Subsequently, it was extensively popularized during almost two decades, every two to three years (Bowles & Koopman, 2007; Bowles & Koopman, 2010; Spiller, Bowles & Koopman, 2012; Feng, Bowles & Koopman, 2014; Spiller & Bowles, 2015; Spiller, Koopman & Bowles, 2017; Spiller & Bowles, 2019; Spiller & Bowles, 2022). The same causes inevitably producing the same effects, organotypic culture experiments and/or expression pattern analyses also exist to support the model in multiple vertebrate species (Childs, Cowan, Kinnell, Anderson & Saunders, 2011; Feng et al., 2015; Lau, Lee & Chang, 2013; Li et al., 2016; Pipek et al., 2013; Rodriguez-Mari et al., 2013; Smith, Roeszler, Bowles, Koopman & Sinclair, 2008; Wallacides, Chesnel, Chardard, Flament & Dumond, 2009). However, conclusions based on treatment of cell lines, organotypic cultures or animals with ATRA or agonists/antagonists have often not been supported by animal studies in which genes encoding for ATRA metabolism enzymes or for ATRA receptors are deleted (Cunningham & Duester, 2015). Accordingly, the model was challenged in 2011 by a first, elegant, genetic study (Kumar et al., 2011), that was the matter of a fiery debate (Griswold, Hogarth, Bowles & Koopman, 2012; Kumar, Cunningham & Duester, 2013). To solve it, some authors called for the analysis of fetal ovaries when all three ALDH1A enzymes or all three ATRA-receptors (RAR) were genetically rendered nonfunctional simultaneously (Agrimson & Hogarth, 2016). In the following section, we discuss the evidence drawn from such mutant mouse models.

## 3. Mutant mouse models disqualify ATRA as the MIS

The first blow to the established model came from the analysis of mutant mice lacking ALDH1A2 or ALDH1A2 and ALDH1A3 (Kumar et al., 2011). The *Aldh1a2*-null mutants die during early embryogenesis, preventing examination of ATRA-dependent processes later in fetal development. To circumvent this lethality, low doses of ATRA were given to pregnant females from E6.25 to E9.5, allowing thereby ALDH1A2-deficient embryos to develop through E14.5. As ATRA is cleared within 12-24 hours (Mic, Haselbeck, Cuenca & Duester, 2002), such a protocol allows the mutant fetuses to reach a developmental stage at which the gonads can be investigated in the absence of ALDH1A2 activity and the absence of ATRA administered several days earlier. Besides, by using the ATRA-sensitive reporter transgene *Tg(RARE-Hspa1b/lacZ)12Jrt* (Rossant, Zirngibl, Cado, Shago & Giguère, 1991), it was demonstrated that the rescued *Aldh1a2*-null fetuses totally lacked ATRA activity in the mesonephros and the ovaries at the time meiosis initiates. But against the odds, normal expression of *Stra8* was detected, indicating meiotic initiation was normal. The same holds true when ALDH1A3 was

simultaneously lacking along with ALDH1A2 in double mutants. Based on these findings it was proposed that *Stra8* expression does not depend on ATRA-signalling, meiotic initiation can take place in the absence of ATRA and even though ATRA is synthesized in the mesonephros, it is totally dispensable to both of these events (Kumar et al., 2011). The proponents of the established model argued that the ALDH1A1 enzyme was present in the ovaries of these mutants, ensuring that some ATRA produced locally acted redundantly to induce meiosis (Griswold, Hogarth, Bowles & Koopman, 2012). In agreement with this proposal, they showed that the expression of *Stra8* and the onset of meiosis were delayed of about 1 day in *Aldh1a1*-null ovaries. They concluded that ALDH1A1 is the main ATRA-synthesizing enzyme in the ovary, and considered as resolved the conundrum posed by the conflicting data sets (Bowles et al., 2016). However, the absence of ATRA-dependent activity in the mesonephros and ovary of mutants lacking both ALDH1A2 and ALDH1A3 (Kumar et al., 2011), casts doubts on the possibility that ALDH1A1 actually generates ATRA for ovary.

In 2020, two other genetic studies put a cat among the pigeons. In the first one, three distinct gene deletion models were generated, in which ablation of *Aldh1a1*, *Aldh1a2* and *Aldh1a3* was simultaneously induced from E9.5, E10.5 or E11.5 (Chassot et al., 2020). Importantly, the efficient loss of ALDH1A activity was assessed by using two sensitive GFP-based reporter systems, and by measuring the residual amount of ATRA by mass spectrometry. These controls showed that the mutant ovaries contained less ATRA than the control testes (Chassot et al., 2020), in which ATRA is supposedly absent, because it is cleared by CYP26B1 (Bowles & Koopman, 2007). The lack of ATRA was also attested by the morphological defects displayed by the fetuses, which were characteristic of a functional state of ATRA-deficiency (Mark, Ghyselinck & Chambon, 2006; Niederreither & Dollé, 2008; Ghyselinck & Duester, 2019). At E13.5, *Stra8* was expressed at a lower level than in controls, but at E16.5 most, if not all, germ cells were meiotic in the ovaries of such mutants. This genetic study shows therefore that deletion of all three *Aldh1a* genes slightly delays but does not impair meiosis entry, indicating that ATRA signaling is dispensable for instructing meiosis in female germ cells (Chassot et al., 2020).

In the second study, another gene deletion model was generated, in which *Rara* and *Rarg* ablation was induced from either E9.5 or E10.5 in the context of mice carrying a *Rarb*-null mutation that can survive and reproduce (Vernet et al., 2020). Efficient loss of RARs was assessed at the gene level by using PCR, at the mRNA level by using *in situ* hybridization, and at the protein level by using immunohistochemistry and western blots with anti-RAR antibodies. The loss of RAR functioning was also attested by the multiple morphological defects displayed by the mutant fetuses, which were, here again, characteristic of a functional state of ATRA-deficiency (Mark, Ghyselinck & Chambon, 2006; Niederreither & Dollé, 2008; Ghyselinck & Duester, 2019). At E14.5, many germ cells expressed STRA8, the cohesin REC8 and the synaptonemal complex protein SYCP3 in the ovaries of these mutants. Moreover, the expression of mRNAs encoding several other meiotic genes was evidenced in individual germ cells devoid of *Rara*, *Rarb* and *Rarg* mRNAs. Among them, *Rec8* mRNA was normally expressed, although it is considered as an ATRA-regulated gene (Koubova et al., 2014). A notable exception was *Stra8* mRNA, whose expression was delayed by 24 hours. Nonetheless, most, if not all, the RAR-null oocytes reached the zygotene stage of meiosis at E15.5 (Vernet et al., 2020). These findings indicate that RARs are dispensable for both ovary differentiation and meiosis initiation during the fetal life. At best, they are instrumental to the timely expression of *Stra8*, which can however recover within 1 day of development. By using grafting experiments, this study further demonstrated that the ovaries of mutants lacking RARs produce functional oocytes which are able to complete meiosis and fertilization postnatally, indicating thereby that the entire meiotic process can take place in the total absence of RARs (Vernet et al., 2020). The fact that ablation of all 3 *Rar*-coding genes in the somatic cells of the female urogenital ridge from E10.5 does not impair granulosa cell specification, differentiation, and reproductive function further demonstrates that ATRA-signaling is dispensable for ovary physiology (Minkina et al., 2017).

Recently, a third genetic study was published aimed at identifying the ATRA-responsive elements (RAREs) controlling expression of *Stra8* (Feng et al., 2021). It showed that subtle mutation of two functional RAREs located upstream of *Stra8* promoter was sufficient to impair the ATRA-dependent expression of a reporter gene in embryonic carcinoma cells *in vitro*. Mutations of the same two sites *in vivo* resulted in the loss of about 50% of *Stra8* expression, but normal expression of many other meiotic

genes at E14.5. Not surprisingly, these RARE mutant females were fertile. The remaining expression of *Stra8* was optimistically ascribed by the authors to other, yet unidentified, RAREs possibly located upstream of the regulatory region they studied (Feng et al., 2021). However, another explanation could be that it rather represented the expression level induced independently of ATRA-activated RARs, as evidenced in RAR- and ALDH1A-deficient ovaries (Chassot et al., 2020; Vernet et al., 2020). In keeping with alternative possibility, deletion of the proximal regulatory region that encompassed a DMRT1-binding site totally abrogated *Stra8* expression both *in vitro* and *in vivo* (Feng et al., 2021).

In the end, the genetic studies altogether point to the following conclusions. First, in rescued *Aldh1a2*- or *Aldh1a2;Aldh1a3*-null mutants (Kumar et al., 2011) meiosis initiation is normal. Second, in mutant females null for *Aldh1a1* alone (Bowles et al., 2016), for all 3 *Aldh1a* simultaneously (Chassot et al., 2020), for all 3 *Rars* simultaneously (Vernet et al., 2020), or in mutants lacking the RAREs of *Stra8* gene (Feng et al., 2021), female germ cells entered meiosis in the fetal ovaries, despite a slightly reduced and delayed expression of *Stra8*. Therefore, we propose a simplest explanation to account for these findings: (i) meiosis initiation *per se* does not depend on ATRA signalling; (ii) ATRA synthesized by ALDH1A1 in the ovary itself activates RAR signaling to amplify the timely expression of STRA8 at E13.5, not to initiate it; (iii) ATRA synthesized in the mesonephros by ALDH1A2 is useless to this process. Accordingly, ATRA should no longer be considered as the long-sought MIS, because meiosis can clearly occur in ovaries devoid of either ATRA or RARs. Quite interestingly, such a conclusion made for oocytes echoes the recent observation that both expression of *Stra8* and initiation of meiosis can also occur in spermatocytes devoid of ATRA (Teletin et al., 2019; Kirsanov et al., 2023). In the end, the reasoning that pharmacological ATRA induces *Stra8* expression in P19 embryonal carcinoma cells (Oulad-Abdelghani et al., 1996), and *Stra8* controls meiotic entry in the mouse (Baltus et al., 2006), implies that ATRA control of meiotic entry looks like a syllogism, which no longer stands up to the genetic facts.

#### 4. How to reconcile seemingly irreconcilable observations

The genetic studies apparently contradict the admitted model (Bowles & Koopman, 2007; Griswold, Hogarth, Bowles & Koopman, 2012), but several clarifications can be proposed to explain the discrepancies. First, the conclusion made from mutant mice that *Stra8* expression and meiotic initiation are not instructed by, but simply facilitated by ATRA is not incompatible with the finding that germ cells failed to enter meiosis in the ovaries of vitamin A-deficient (VAD) female rat fetuses (Li & Clagett-Dame, 2009). In this model, *Stra8* expression is reduced but not abolished at E15.5, and the number of SYCP3-positive oocytes is decreased but not absent at E18.5, and the germ cells that do enter meiosis progress through the meiotic prophase until E21.5 (Li & Clagett-Dame, 2009). In rat ovaries, the onset of meiosis is much less synchronous than in mice. Only 10% of germ cells enter the preleptotene stage between E18 and E18.5 (Hilscher & Hilscher, 1972; Prépin, Gibello-Kervran, Charpentier & Jost, 1985). The proportion of meiotic cells raises to 75% E19.5 and 100% at E21.5, with still 10% of oocytes being in leptotema (Prépin, Gibello-Kervran, Charpentier & Jost, 1985). Thus, one can easily imagine that meiosis was delayed by VAD, instead of being arrested, as are most of the other morphogenetic vitamin A-dependent processes in VAD rats (reviewed in Mark, Ghyselinck & Chambon, 2006). In keeping with this interpretation, some germ cells still expressed the PGC marker POU5F1 in the ovaries of VAD rats at E18.5, indicating that their differentiation is delayed (Li & Clagett-Dame, 2009). Analyzing VAD ovaries between E19.5 and E20.5 may have revealed that germ cells indeed entered meiosis, but later than in vitamin A-sufficient conditions. Along the same vein, the fact that treatment of E11.5 mouse urogenital ridges for 48 hours with the ALDH1A inhibitor WIN18,446 (Paik et al., 2014) reduced, but did not abolish, *Stra8* expression means by no way that meiosis does not initiate in ATRA-deficient ovaries (Hogarth et al., 2011). Here again, one can propose that meiosis was delayed by WIN18,446, instead of being arrested.

Second, the experiments done to prevent ATRA or RAR functioning in cultured fetal ovaries were all performed by using BMS-204493 (Koubova et al., 2006; Koubova et al., 2014) or AGN193109 (Bowles et al., 2006; Bowles et al., 2016), which are not RAR neutral antagonists, but actually RAR inverse agonists. Such ligands are capable of repressing RAR basal activity by favoring and stabilizing recruitment of corepressors at RARE-containing loci, even in the absence of endogenous ATRA (Klein

et al., 1996; Germain et al., 2009). As the *Stra8* promoter contains RAR-binding sites on which RARs are actually bound *in vivo* (Kumar et al., 2011; Chatagnon et al., 2015; Feng et al., 2021), adding a RAR inverse agonist necessarily tethers nuclear receptor corepressors at the locus, promotes chromatin compaction and thereby *Stra8* extinction, irrespective of the presence of endogenous ATRA in the ovary. The same holds true for the *Rec8* gene, which also contains a RARE (Chatagnon et al., 2015). The fact that expression of these two genes is shut down upon exposure to the RAR inverse agonists therefore means by no way that it is normally controlled by endogenous ATRA. The use of a RAR neutral antagonist would have been much more informative (Germain et al., 2009). Then, assuming that *Rec8* and *Stra8* are actually not regulated by endogenous ATRA, it is not surprising to find them expressed in ovaries lacking ATRA (Chassot et al., 2020) or RARs (Vernet et al., 2020).

Third, it has been emphasized that alterations generated by exogenously administered ATRA at high levels do not necessarily reflect physiological processes operating in real life with endogenous ATRA which exists at a much lower level (Horton & Maden, 1995; Mark, Ghyselinck & Chambon, 2006). However, the paradigm according to which the physiological effects of a substance can be identified by studying the consequences of an excess of that substance administered to living beings is still alive and kicking in biology. By comparison, it's hard to imagine researchers in economics or in social sciences determining the purchasing power of a population by studying the behavior of lottery winners. Yet this is exactly the kind of approach biologists are using, literally flooding organs or cells with huge, non-physiological doses of ATRA, in order to determine the effects of endogenous ATRA, that normally acts at very low doses and only in given cell-types. As a matter of fact, conclusions based on treatment of cell lines or animals with ATRA, agonists or inverse agonists have often not been supported by animal studies in which genes encoding ALDH1As or RARs are deleted (Cunningham & Duster, 2015). In the case of the gonads, the simple fact that RAREs are present in *Stra8* gene can explain its artefactually forced expression by ATRA or agonists added to gonads cultured *in vitro* (Bowles et al., 2006; Koubova et al., 2006). Then, one could ask why the *Stra8* gene contains RAREs if its expression does not need to be induced by ATRA for meiotic initiation? The sole situation in which *Stra8* is actually induced by ATRA *in vivo* appears to be prior to meiosis during spermatogonia differentiation, at the transition between the A aligned (undifferentiated) and A1 (differentiated) states (Zhou et al., 2008; Busada & Geyer, 2016; Griswold, 2022). Ironically, the role of STRA8 in this process is largely unknown.

## 5. What else, if not ATRA?

Since ATRA cannot be the MIS, entering meiosis is either an autonomous, intrinsic, property of female germ cells (discussed in Kocer, Reichmann, Best & Adams, 2009), or it requires one or several other extrinsic cues acting as a MIS. As to the intrinsic properties of germ cells, expression of the RNA-binding protein DAZL is key for their acquisition of competence (also called licensing) to undergo either the female- or the male-specific path of differentiation (Lin, Gill, Koubova & Page, 2008; Gill, Hu, Lin & Page, 2011). In addition, the DMRT1 transcription factor contributes to the switch from mitosis to meiosis by directly regulating *Stra8* expression in female germ cells (Krenz et al., 2011). Then, some epigenetic factors are also instrumental to the expression of *Stra8*, and consequently, the initiation of meiosis (Wang & Tilly, 2010). Examples of such factors are (i) the DNMT1 and TET1 enzymes, that catalyze DNA methylation and demethylation of meiotic genes at specific stages, respectively (Yamaguchi et al., 2012; Hargan-Calvopina et al., 2016), and (ii) the polycomb repressive complex PRC1 or the SWI/SNF complex, that promote structural modifications of chromatin (Yokobayashi et al., 2013; Ito et al., 2021).

As to the extrinsic cues, recent data showed that vitamin C is required during gestation to induce DNA demethylation of key genes in germ cells, and therefore the timed initiation of meiosis (DiTroia et al., 2019). The WNT/ $\beta$ -catenin (CTNNB1) signaling pathway also plays a critical role in meiosis because a proportion of PGCs neither expressed *Stra8* nor entered meiosis in the absence of RSPO1, an activator of this pathway in the fetal ovary (Chassot et al., 2011). Signaling by bone morphogenetic proteins (BMPs) is another pathway instrumental to meiotic initiation. In female PGC-like cells derived from embryonic stem cells, ATRA and STRA8 are not sufficient on their own to induce premeiotic DNA replication, unless BMPs are added, indicating that ATRA signaling serves to enhance a

preexisting situation (Miyauchi et al., 2017). This finding is tied with earlier studies, showing that ATRA accelerated entry in meiosis in cultured rat fetal ovaries (Livera, Rouiller-Fabre, Valla & Habert, 2000). In response to BMP signaling, (i) the MSX1 and MSX2 homeodomain transcription factors activate *Stra8* expression and promote thereby meiosis initiation in female germ cells (Le Bouffant et al., 2011); and (ii) the transcriptional regulator with GATA-like zinc fingers ZGLP1 activates key genes involved in meiosis initiation, independently of ATRA (Nagaoka et al., 2020). In response to activin A (INHBA homodimers), expression of STRA8 was increased and progression of meiosis was accelerated (Liang et al., 2015). Together, these studies indicate that several convergent pathways are able to initiate *Stra8* expression and the transition from mitosis to meiosis cycle, independently of ATRA signaling.

## 6. Mutant mouse models support the idea that CYP26B1 acts as an MPS

While the action of ATRA in the fetal ovary is contested, there is unquestionable genetic evidence that CYP26B1 is a testis-specific factor acting either by promoting male germ cells to enter a transient mitotic arrest in G0/G1 phase of cell cycle (quiescence) between E12.5 and E14.5, or by preventing them from entering meiosis during fetal development. The analysis of fetal testes from a first *Cyp26b1*-null mouse line (Yashiro et al., 2004) actually showed expression of *Stra8* et *Sycp3* at E13.5, indicating that germ cells entered meiotic prophase I at an early stage of development (Bowles et al., 2006). This finding was extended by the analysis of two other *Cyp26b1*-null mouse lines, showing that germ cells entered meiosis between E13.5 and E15.5: they expressed *Rec8*, *Sycp3*, *Dmc1* and *Spo11* mRNAs as well as STRA8 protein, and proceeded through meiosis until the zygotene or pachytene stages of prophase I (MacLean, Li, Metzger, Chambon & Petkovich, 2007; Bowles et al., 2010; Koubova et al., 2014). This phenotype is not due to a sex-reversal of the testicular somatic environment, because both Sertoli and Leydig remained normally differentiated, as attested by their expression of anti-Müllerian hormone (AMH) and 3 $\beta$ -hydroxysteroid dehydrogenase (HSD3B1), respectively (MacLean, Li, Metzger, Chambon & Petkovich, 2007). In addition, starting from E13.5 a number of germ cells were lost by apoptosis in *Cyp26b1*-null testes, as attested by terminal deoxynucleotidyl transferase dUTP nick-end labelling and cleaved caspase-3 staining (MacLean, Li, Metzger, Chambon & Petkovich, 2007). As ablation of *Cyp26b1* in Sertoli cells induces a similar outcome, it can be concluded that CYP26B1 acts in Sertoli cells, but not in interstitial cells (Li, MacLean, Cameron, Clagett-Dame & Petkovich, 2009).

By deleting *Cyp26b1* specifically in Sertoli cells by E15.5, it was additionally shown that abolishing CYP26B1 activity caused male germ cells to exit from G0, to re-enter the mitotic cycle, and then to initiate the meiotic prophase (Li, MacLean, Cameron, Clagett-Dame & Petkovich, 2009). In keeping with this, it was shown that pharmacological inhibition of CYP26B1 activity in cultured testes prevented male germ cell mitotic arrest, and allowed commitment to the male program of development (Trautmann et al., 2008). Later, it was further shown that CYP26B1 suppresses two distinct programs in male germ cells: a STRA8-dependent meiotic pathway and a STRA8-independent mitotic pathway (Saba, Wu & Saga, 2014). As CYP26B1 currently has no other known substrates than ATRA (Topletz et al., 2012; Thatcher & Isoherranen, 2009; Isoherranen & Zhong, 2019), the admitted idea is that ATRA is the trigger for the defects displayed by *Cyp26b1*-null testes. It is hypothesized that CYP26B1 acts by forming a catabolic barrier which prevents the exposure of male germ cells to ATRA. However, is the current evidence that ablation of CYP26B1 increases ATRA in the fetal testis solid?

## 7. The evidence that *Cyp26b1* ablation increases testis ATRA is all refutable

First, experiments showed that *Stra8* expression was not induced when fetal testes were cultured in the presence of a potent, but non-specific, CYP inhibitor (ketoconazole), combined with the RAR inhibitor BMS-204493. The same effect was observed when a more selective inhibitor of CYP26s (Ro115866) was tested in combination with BMS-204493 (Koubova et al., 2006). Assuming that *Stra8* expression is necessarily controlled by ATRA (see above), these findings supposedly constitute the crux demonstrating that ATRA was present in fetal testes when CYP26B1 was inhibited. However, as discussed above, BMS-204493 is not a RAR neutral antagonist, but a RAR inverse agonist, which does not compete with endogenous ATRA but represses RAR activity, even the absence of ATRA (Germain et al., 2009). This casts serious doubts on the conclusion that ATRA levels were actually increased upon inhibition of CYP26B1. In keeping with this, the finding that administration of ketoconazole was unable

to increase activity of the *Tg(RARE-Hspa1b/lacZ)12Jrt* transgene in control fetal testes led to the conclusion that ATRA degradation is not the mechanism by which CYP26B1 acts (Kumar et al., 2011).

Second, because the germ cells of wild-type fetal testes treated with Am580, an RAR agonist that is resistant to the action of CYP26B1, promptly expressed *Stra8* and entered meiosis, it was concluded that ATRA, rather than another metabolite synthesized by CYP26B1, was responsible for phenotypes observed in *Cyp26b1*-null testes (MacLean, Li, Metzger, Chambon & Petkovich, 2007). This was, however, not unexpected, given that treating wild-type fetal testes with either ATRA or RAR agonists selective for each of the three RAR isotypes (i.e., BMS-194753 for RARA, BMS-213309 for RARB and BMS-270394 for RARG) similarly activated *Stra8* expression and meiotic initiation (Koubova et al., 2006). If the correct interpretation of such experiments is that CYP26B1 was not clearing the testes from synthetic ligands because they are resistant to CYP26B1-mediated metabolism (MacLean, Li, Metzger, Chambon & Petkovich, 2007; Bowles & Koopman, 2007; Griswold, Hogarth, Bowles & Koopman, 2012), why would ATRA treatment produce exactly the same results although it is sensitive to CYP26B1-mediated metabolism (Bowles et al., 2006; Koubova et al., 2006)?

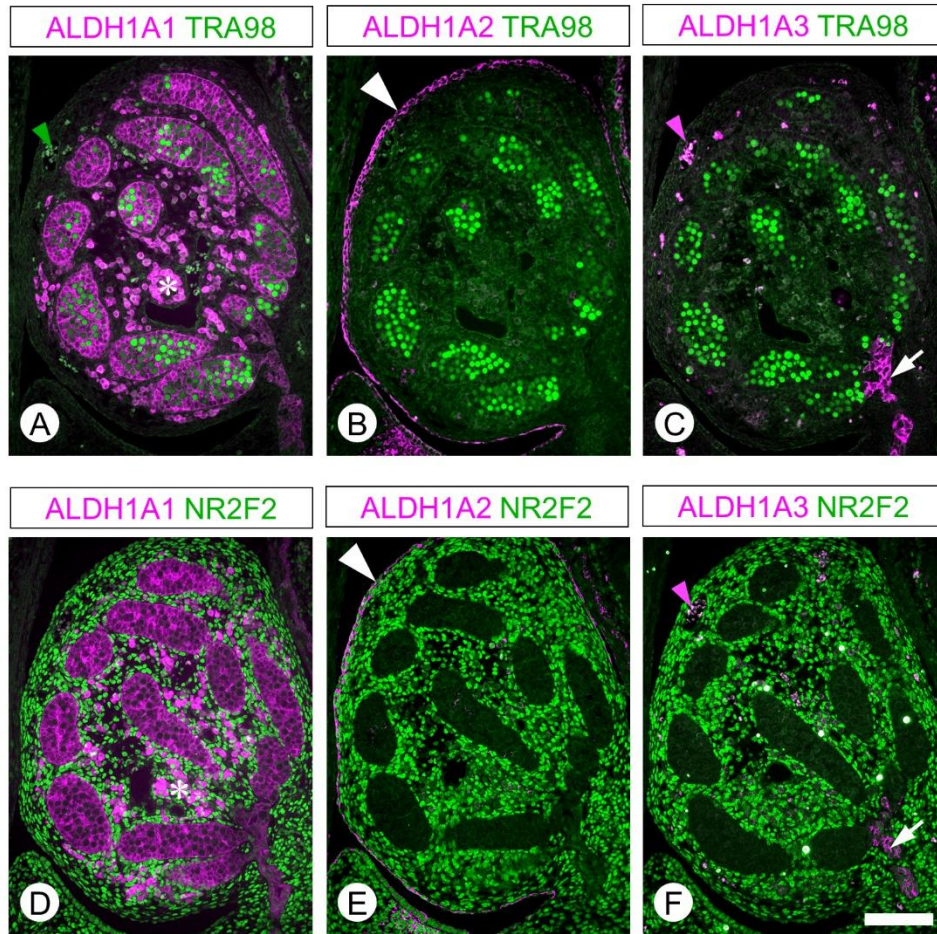
Third, on the basis of ATRA-dependent reporter cells cultured with testicular extracts, *Cyp26b1*-null fetal testes were shown to contain three-times more ATRA than control testes. However, the absolute quantities detected in each extract were not specified (MacLean, Li, Metzger, Chambon & Petkovich, 2007). This should not represent large quantities, since control testes are devoid of ATRA, based on the activity of the *Tg(RARE-Hspa1b/lacZ)12Jrt* transgene (Bowles et al., 2006; Kumar et al., 2011). Consistent with this, the amount of ATRA measured in control testes was almost equal to the amount of ATRA remaining in the ovaries lacking *Aldh1a1*, *Aldh1a2* and *Aldh1a3* from E10.5 (Chassot et al., 2020). In the end, three times little is still little, or nothing at all.

Fourth, the expression of *Aldh1a1* is three-fold lower in *Cyp26b1*-null testes than in controls (Bowles et al., 2009; see also below). Knowing that ATRA cannot originate from the mesonephros (Kumar et al., 2011), this decrease of *Aldh1a1* expression rather evokes a decreased production of ATRA in the seminiferous cords of *Cyp26b1*-null mutants.

## 8. Meiosis initiates in CYP26B1-deficient testes lacking ALDH1A1

These considerations collectively suggest that ATRA degradation may not be the mechanism underlying CYP26B1 action in the fetal testis. To expand on this provocative notion, we thought it would be interesting to study the fate of germ cells in testes lacking both CYP26B1 and ATRA-synthesizing enzymes. We first determined by immunohistochemistry the expression pattern of ALDH1A1, ALDH1A2 and ALDH1A3 at E15.5, the developmental stage when STRA8 protein expression is at its climax in *Cyp26b1*-null testes (Koubova et al., 2014). The antibody against ALDH1A1 gave a robust signal in the cytoplasm of Sertoli cells surrounding the germ cells (Fig. 1A, D), and in some NR2F2-negative cells of the interstitial tissue (asterisks, Fig. 1A, D). The expression of ALDH1A2 was limited to the surface epithelium lining the testis (large arrowheads, Fig. 1B, E), while that of ALDH1A3 was restricted to the Sertoli cells of the *rete* testis region (arrows, Fig. 1C, F). This pattern is essentially similar to the one observed at E14.5 (Teletin, Vernet, Ghyselinck & Mark, 2017). As it is unlikely that ATRA acts as a long-distance diffusing molecule to reach the germ cells, which are encapsulated in the seminiferous cords, we reasoned that a null mutation of *Aldh1a1* was sufficient to make the environment of germ cells devoid of ATRA. Thus, we generated *Cyp26b1*<sup>-/-</sup>;*Aldh1a1*<sup>-/-</sup> compound mutant fetuses and analyzed their testes.

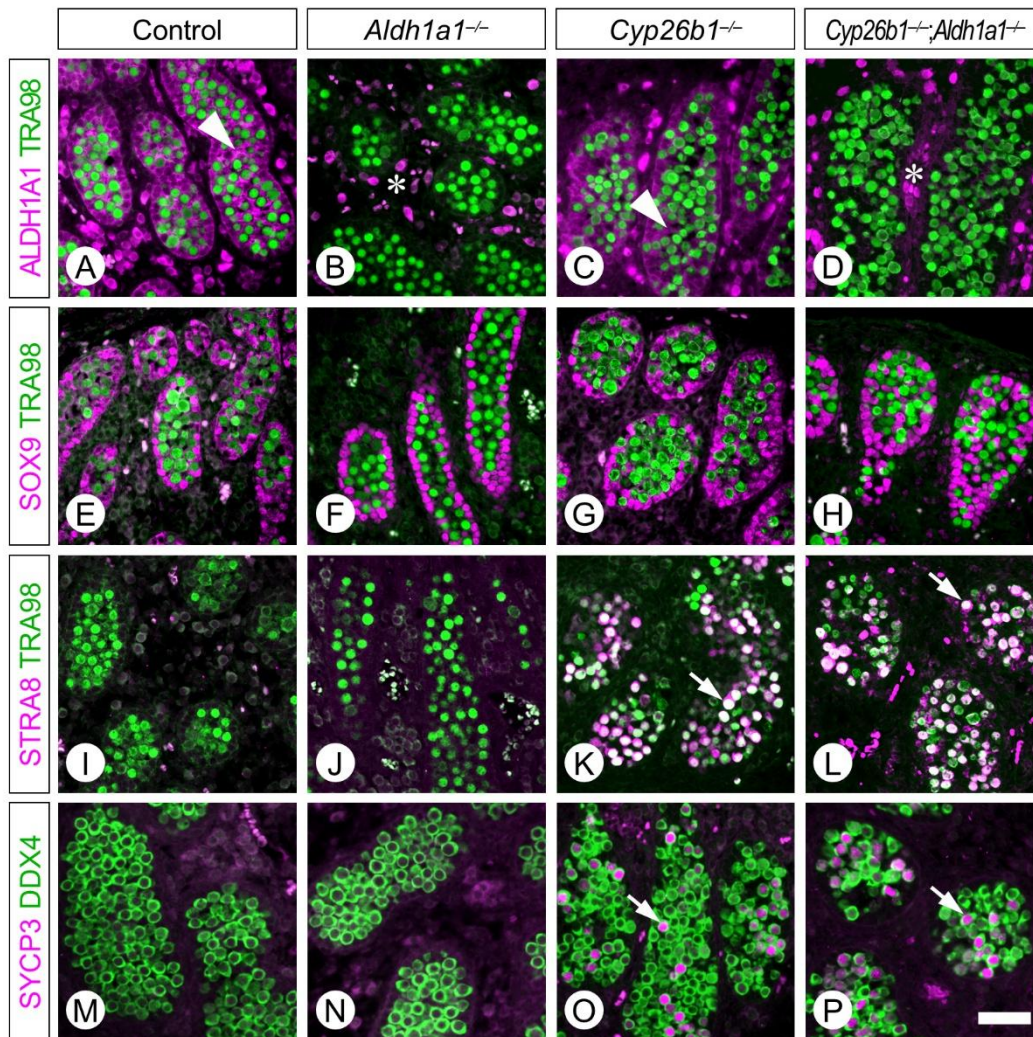
In the control testis at E15.5, ALDH1A1 was detected in Sertoli and interstitial cells (arrowhead, Fig. 2A). The signal in Sertoli cells was no longer detected in the *Aldh1a1*<sup>-/-</sup> background (Fig. 2B, D), while it persisted in the interstitial cells (asterisks). The signal in these cells may correspond to either a non-specific staining, or to ALDH1A7, whose peptide sequence displays 90% identities with ALDH1A1 (Hsu, Chang, Hoffmann & Duyster, 1999) and whose mRNA is expressed in fetal testes (Bowles et al., 2009). This enzyme cannot however contribute to the amount of ATRA build up in the fetal testis because it does not synthesize ATRA (Hsu, Chang, Hoffmann & Duyster, 1999). The ALDH1A1 protein was detected at a lower level in Sertoli cells of the *Cyp26b1*<sup>-/-</sup> testis (arrowhead, Fig. 2C), in agreement with the reported decreased level of *Aldh1a1* mRNA in *Cyp26b1*-null testes (Bowles et al., 2009).



**Figure 1. The major ATRA-synthesizing enzyme expressed in the fetal testis and contacting germ cells is ALDH1A1.** Transverse, consecutive, histological sections of E15.5 fetuses immunolabeled with antibodies against ALDH1A1 (A, D), ALDH1A2 (B, E) or ALDH1A3 (C, E; magenta signals) and TRA98 (A–C), a nuclear protein specifically expressed in germ cells, or NR2F2 (D–F), a nuclear receptor formerly called COUPTF-II (green signals). ALDH1A1 is restricted to the Sertoli cells, which are encapsulating germ cells, and to some somatic cells, which were NR2F2-negative and located in the interstitial tissue of the testis (asterisks). ALDH1A2 is restricted to the surface epithelium of the testis (large arrowheads). ALDH1A3 is restricted to the rete testis (arrows). Red blood cells are sometimes artefactually stained in green (small green arrowhead in A) or in magenta (small magenta arrowhead in C and F). Scale bar in (F): 100  $\mu$ m (A–F).

Importantly, Sertoli cells remained normally differentiated in the absence of CYP26B1, singly or in combination with loss of ALDH1A1. This was assessed by detecting SOX9, which appeared unchanged between control and mutant testes (Fig. 2E–H). This indicated that the somatic environment of the germ cells did not experience ATRA-induced sex-reversal, contrasting thereby with the observation that the *Cyp26b1*<sup>-/-</sup> testis displayed a mild ovotestis phenotype at E13.5 (Bowles et al., 2018). Then, as previously shown (MacLean, Li, Metzger, Chambon & Petkovich, 2007; Koubova et al., 2014), numerous germ cells were STRA8-positive and/or SYCP3-positive in the testis of *Cyp26b1*<sup>-/-</sup> mutants at E15.5 (arrows, Fig. 2K, O), indicating they were meiotic. In contrast, none of them initiated meiosis in control and *Aldh1a1*<sup>-/-</sup> testes (Fig. 2I, J, M, N). Most importantly, numerous germ cells were STRA8-positive and/or SYCP3-positive also in the testis of *Cyp26b1*<sup>-/-</sup>;*Aldh1a1*<sup>-/-</sup> compound mutants (Fig. 2L, P), in proportions similar to that observed in *Cyp26b1*<sup>-/-</sup> mutants (compare Fig. 2K with 2L, and 2O with 2P). Considering that ALDH1A1 is the major source of ATRA in the fetal testis (see above), this indicates that the defects displayed by the CYP26B1-deficient testes cannot be ascribed to an excess of uncleared ATRA because they are not rescued by ablation of ALDH1A1. We may anticipate that the proponents of the established model will cry foul because ALDH1A2 and ALDH1A3 were still present in *Cyp26b1*<sup>-/-</sup>;*Aldh1a1*<sup>-/-</sup> mutants. However, we could argue that ALDH1A1 is the sole, functional, source of ATRA in the fetal ovary (see above), despite the expression of ALDH1A2 in the coelomic epithelium (Teletin, Vernet, Ghyselinck & Mark, 2017). Therefore, there is no obvious reason that a

similar expression pattern of ALDH1A2 in the surface epithelium of the fetal testis (Fig. 1) may provoke a different effect. In the end, the present genetic study and data from two other studies (Kumar et al., 2011; Bellutti et al., 2019) support the notion that CYP26B1 does not prevent germ cells to enter meiosis by degrading ATRA in the fetal testis.



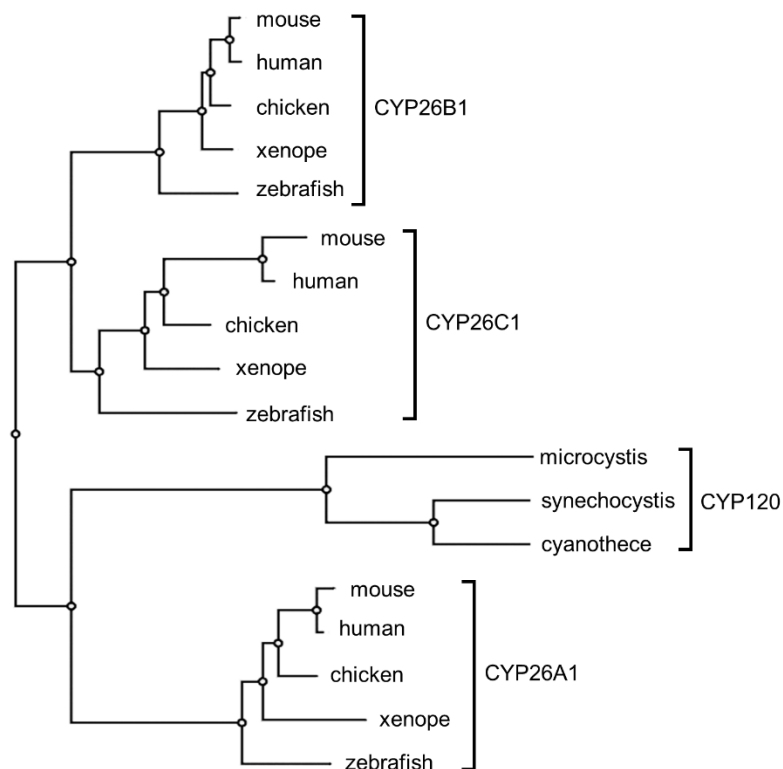
**Figure 2. Germ cells are meiotic in the testis of mouse fetuses lacking either CYP26B1 alone or CYP26B1 and ALDH1A1 together.** Transverse histological sections of E15.5 fetuses immunolabeled with antibodies against ALDH1A1 (A–D), SOX9 (E–F), STRA8 (I–L) or SYCP3 (M–P; magenta signals) and TRA98 (A–L), a nuclear protein specifically expressed in germ cells, or DDX4 (M–P), a cytoplasmic ATP-dependent RNA helicase specific to germ cells (green signals). ALDH1A1 is restricted to the Sertoli cells (arrowheads), which are encapsulating germ cells (A). Note that intensity of the Sertoli cell signal is reduced in the CYP26B1-deficient testis (C). The signal observed in some somatic cells located in the interstitial tissue of the testis (asterisks) is unspecific as it is detected also in the ALDH1A1-deficient testis (B, D). SOX9 is detected in the nuclei of Sertoli cells of both control and mutant testes (E–H). STRA8 is not detected in control and in ALDH1A1-deficient testes (I, J). In contrast, it is detected in a large proportion of germ cell nuclei (arrows) in mutants lacking CYP26B1 alone, or lacking both CYP26B1 and ALDH1A1 (K, L). The superimposition of magenta and green signals makes the germ cells appearing white (K, L). SYCP3 is not detected in control and in ALDH1A1-deficient testes (M, N). In contrast, it is detected in several germ cell nuclei (arrows) in mutants lacking CYP26B1 alone, or lacking both CYP26B1 and ALDH1A1 (O, P). Scale bar in (P): 50  $\mu$ m (A–P).

Consistent with this proposal, it was recently shown that the ectopic expression of CYP26B1 in fetal ovaries significantly decreased the number of STRA8-positive germ cells. In contrast, the ectopic expression of CYP26A1 was unable to abolish *Stra8* expression and to impair meiotic initiation (Bellutti et al., 2019). Considering that CYP26A1 is much more (at least 10-fold more) potent than CYP26B1 to degrade ATRA (Toplez et al., 2012; Isoherranen & Zhong, 2019), it is evident that clearing ATRA efficiently had no effect on meiotic initiation (Bellutti et al., 2019). This finding further confirms that

ATRA is not the signal initiating meiosis in the fetal ovary (see above), and indicates that CYP26B1 displays an activity other than degrading ATRA in the fetal testis (Bellutti et al., 2019).

### 9. If it does not degrade ATRA, what does CYP26B1 do?

Instead of degrading ATRA, CYP26B1 likely functions either by degrading an as yet unknown MIS derived from the mesonephros, or by synthesizing an MPS in the fetal testis. The former hypothesis is supported by explant studies suggesting that a factor other than ATRA travels from the mesonephros to the testis to induce meiosis (Kumar et al., 2011). The latter hypothesis finds support from older studies suggesting the existence of a meiosis-inhibiting factor produced in the fetal testis (McLaren & Southee, 1997). This, of course, does not fit to CYP26B1 itself, since it is a cytoplasmic enzyme located in Sertoli cells. It may only apply to one of its substrates or products, respectively, that can diffuse to the germ cells.



**Figure 3. CYP26 enzyme phylogenetic tree constructed with protein sequences.** All CYP26 protein sequences were obtained from NCBI protein database. Evolutionary analyses were conducted in MAFFT alignment. The evolutionary history was inferred using the UPGMA method (Sneath & Sokal, 1973) and the evolutionary distances were computed using the Poisson correction method (Zuckerkandl & Pauling, 1965).

During evolution, the phylogenetic separation caused diversification of CYP26A1 from CYP26B1 and CYP26C1, resulting in two independent clades within the CYP26 family, and an additional gene duplication led to separation of CYP26B1 and CYP26C1 genes (Carvalho et al., 2017). Since CYP120 is also able to catalyze the hydroxylation of ATRA in cyanobacteria (Ke, Baudry, Makris, Schuler & Sligar, 2005; Alder, Bigler, Werck-Reichhart & Al-Babili, 2009), we introduced CYP120 of three different species in the phylogenetic tree. Surprisingly, they all clustered with CYP26A1 (Fig. 3), suggesting that the ancestral enzyme for ATRA metabolism is CYP26A1 rather than CYP26B1 or CYP26C1. It is worth noting that within a given species, sequence conservation among the three CYP26 family members is lower than conservation of a given CYP26 member between two species. For instance, mouse CYP26B1 and CYP26A1 only share 42% amino acid sequence identity, while mouse and human CYP26B1 share 97% amino acid sequence identity (MacLean et al., 2001). Nonetheless, all three enzymes are characterized as ATRA hydroxylases that carry out essentially the same metabolic function. Such a functional redundancy is quite unusual with CYP enzymes. For example, CYP19,

CYP17 and CYP24 that conduct endogenous metabolism of a single substrate lack multiple family members. On the opposite, small differences in protein sequence, such as those observed between CYP3A4 and CYP3A5 or between CYP2C9, CYP2C19 and CYP2C8, result in distinct substrate specificities and catalytic activities (Isoherranen & Zhong, 2019). It can be speculated that multiple CYP26 enzymes are required to metabolize different retinoids. Alternatively, it is well possible that CYP26B1 also uses substrates other than retinoids. In keeping with this alternative proposal, CYP26B1 is much less efficient than CYP26A1 to clear ATRA (Topletz et al., 2012; Isoherranen & Zhong, 2019). Moreover, the developed homology models based on other CYP crystal structures suggested some differences in the active site architecture between CYP26A1 and CYP26B1, opening possibilities for other substrates (Karlsson, Strid, Sirsjö & Eriksson, 2008). Last, it is assumed that CYP26B1 needs to interact with ATRA-bound cellular retinoic acid-binding protein 1 (holo-CRABP1) to accept ATRA as a substrate via substrate channeling (Nelson et al., 2016), but CRABP1 is not expressed in fetal Sertoli cells (Souali-Crespo et al., 2023). Further studies are needed to provide insight on the nature and molecular identity of the actual substrates and products of CYP26B1 in the fetal testis.

Aside from a CYP26B1-degraded substrate or a CYP26B1-synthesized product, other signals can evidently prevent meiosis initiation. Treatment of mouse fetal testes with an inhibitor of secretion and post-Golgi trafficking (brefeldin A) was sufficient to induce germ cells enclosed within the testis cords from entering meiosis, indicating that secreted molecules are important to prevent meiosis initiation (Best, Sahlender, Walther, Peden & Adams, 2008). In agreement with this proposal, conditioned media from cultured fetal mouse testes were shown to contain secreted protein(s) capable of inhibiting meiosis, having a molecular weight greater than 10kDa (Byskov, 1978; Guerquin et al., 2010). Accordingly, several secreted proteins are known to prevent meiotic initiation, amongst which is FGF9 (Barrios et al., 2010; Bowles et al., 2010), NODAL (Souquet et al., 2012; Wu et al., 2013), activin and transforming growth factor beta (Miles et al., 2013; Gustin, Stringer, Hogg, Sinclair & Western, 2016). It is however difficult to dissociate whether some of these proteins actually inhibit meiosis or induce mitotic arrest (Moreno et al., 2010; Spiller & Bowles, 2019; Shimada & Ishiguro, 2023).

## 10. What about ATRA in the human fetal gonad?

As to the human fetal gonads, genetic loss-of-function studies are not feasible. Most of the knowledge on the role of ATRA is therefore acquired by using *in vitro* cultures, with the possible pitfalls described above. Nonetheless, it appears that ATRA has no major role in the human gonad, neither in the female nor in the male. On the one hand, no meiotic cells are detected in human fetal testes treated with ATRA (Lambrot et al., 2006). In keeping with this finding, exogenous ATRA induces STRA8 expression in cultured human fetal testis, but is not sufficient to cause expression of meiosis-associated genes (Childs, Cowan, Kinnell, Anderson & Saunders, 2011). Thus, as in the mouse, mechanisms other than CYP26B1-mediated degradation of ATRA may exist to inhibit meiotic initiation in the human fetal testis. On the other hand, many germ cells never initiate meiosis in cultured human fetal ovaries stimulated by ATRA, suggesting that a meiotic inhibitor, unrelated to ATRA, exists in the human ovary (Le Bouffant et al., 2010). Besides, CYP26B1, whose expression is greater in the human fetal ovary than in testis (Childs, Cowan, Kinnell, Anderson & Saunders, 2011), and FGF9 are both present in the human ovary, where they act simultaneously to repress STRA8 and meiosis in human fetal female germ cells (Frydman et al., 2017). Thus, contrary to the mouse, the human fetal ovary and testis share meiotic preventing pathways.

## 11. Concluding remark

The use of genetic loss-of-function approaches is essential to identify the processes controlled by a given gene product (Lloyd, 2024; Gridley & Murray, 2022). Global knockouts or recombinase-mediated cell-type restricted ablation of genes coding for the ATRA-synthesizing enzymes (ALDH1As), the ATRA-receptors (RARs) or the ATRA-degrading enzymes (CYP26s) have been particularly useful for discovering the events regulated by ATRA during embryogenesis and fetal development. One may complain that several pitfalls, such as inconsistent, incomplete or mosaic patterns of deletion, can yield confounding interpretations of the results (Spiller & Bowles, 2022). In the case of RARs, however, effective gene invalidation is indisputable, since (i) RARs are no longer detectable at the protein level, (ii) transcriptome analysis shows that single germ cells no longer contain

mRNA coding for RARs, and (iii) the resulting overall fetal phenotype corresponds in every respect to a state of functional ATRA deficiency (Vernet et al., 2020). In the case of ALDH1A, loss of ATRA-synthesizing activity is also unquestionable since GFP-based reporter systems and quantitative mass spectrometry showed that mutant ovaries contained less ATRA than control testes, in which ATRA is supposedly absent (Chassot et al., 2020). In opposition to the genetic loss-of-function studies dealing with endogenous ATRA, it has been emphasized that studies using administration of exogenous ATRA do not necessarily reflect physiological processes operating in real life (Horton & Maden, 1995; Mark, Ghyselinck & Chambon, 2006). A recent example is treatment with pharmacological levels of ATRA can alter proximo-distal (PD) patterning during limb regeneration, while endogenous ATRA is not normally required for PD patterning as shown by genetic studies that remove ATRA-synthesizing enzymes (Cunningham & Duester, 2015; Berenguer & Duester, 2023). Evidently, most RAREs located in the genome will respond to pharmacological levels of ATRA and induce ectopic activation or repression of nearby genes (Chatagnon et al., 2015), but such a response does not mean that the genes are regulated the same way by endogenous ATRA which is much lower in concentration. In order to determine whether ATRA normally controls a given gene, one must remove endogenous ATRA, preferably by a genetic loss-of-function approach (Duester, 2017). According to such studies described above (Kumar et al., 2011; Chassot et al., 2020; Vernet et al., 2020; Feng et al., 2021), it is legitimate to propose that ATRA-activated RARs participate to the timed expression of STRA8 in female germ cells, but are dispensable for instructing meiosis initiation. Thus, it cannot be ‘the’ meiosis-inducing substance, and the controversy should end.

As to CYP26B1, genetic evidence undoubtedly shows that it is a testis-specific factor acting by preventing male germ cells from entering meiosis during fetal development (Bowles et al., 2006; MacLean, Li, Metzger, Chambon & Petkovich, 2007; Bowles et al., 2010; Koubova et al., 2014; Li, MacLean, Cameron, Clagett-Dame & Petkovich, 2009). However, instead of doing so by degrading ATRA, as widely admitted, CYP26B1 functions either by degrading an as yet unknown ‘meiosis-inducing substance’ derived from the mesonephros, or by synthesizing an equally unknown ‘meiosis-preventing substance’ in the fetal testis.

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