Germline stem cells are critical for sexual fate decision of germ cells

Minoru Tanaka†

Egg or sperm? The mechanism of sexual fate decision in germ cells has been a long-standing issue in biology. A recent analysis identified foxl3 as a gene that determines the sexual fate decision of germ cells in the teleost fish, medaka. foxl3/Foxl3 acts in female germline stem cells to repress commitment into male fate (spermatogenesis), indicating that the presence of mitotic germ cells in the female is critical for continuous sexual fate decision of germ cells in medaka gonads. Interestingly, foxl3 is found in most vertebrate genomes except for mammals. This provides the interesting possibility that the sexual fate of germ cells in mammals is determined in a different way compared to foxl3 Possessing vertebrates. Considering the fact that germline stem cells are the cells where foxl3 begins to express and sexual fate decision initiates and mammalian ovary does not have typical germline stem cells, the mechanism in mammals may have been co-evolved with germline stem cell loss in mammalian ovary.

Keywords: fish; germ cells; mammals; sex; stem cells

Introduction

Recent studies have shown that vertebrates employ various genes for sex determination [1]. Sry, located on the mammalian Y chromosome, was the first sex determination gene identified in vertebrates [2, 3]. Since then, several critical factors have been identified such as Sox9, Fgf9, and Dmrt1 [4]. However, contrary to the initial prediction that Sry is conserved among vertebrates, many animals do not possess an Sry homolog. Ten years after the discovery of Sry, DMY/Dmrt1bY was identified as the sex determination gene on the sex chromosome in the teleost fish, medaka [5, 6]. Since this discovery, other sex determination genes have also been identified in various vertebrates.

Regardless of these variations of the sex determination genes, the first cell type to display sexual discrimination during embryogenesis appears to be conserved among all vertebrates. All sex determination genes examined thus far are expressed in the somatic (supporting) cells that directly surround the germ cells in the gonad [3–12]. Therefore, it is reasonable to speculate that the sexual fate of germ cells (in other words, the fate decision of germ cells to develop eggs or sperms) is triggered by the sex of the surrounding somatic cells during a normal sex determination process. Thus, the precise timing and mechanism of germ cell sexual fate determination by somatic cells needs to be assessed.

The precise molecular mechanism underlying germ cell sexual fate decision is yet to be determined. However, a few studies on the cellular level have provided clues as to the mechanism. In a mouse ex vivo culture study, germ cells isolated from male gonad at 12.5 dpc (days post-coitum) maintained the male characteristics even when cultured in the presence of only female somatic cells, suggesting that the fate decision of germ cells to male occurs by around 12.5 dpc, 2 days after the onset of Sry expression in the supporting cells. XX germ cells do not exhibit any sign of meiosis at 12.5 dpc, but they do at 13.5 dpc in a culture condition where male gonadal primordial cells were present. Therefore, 13.5 dpc was determined as the time when the decision to female is made [13, 14].

Consistent with the results of ex vivo culture experiments, several factors—including Fgf9 and retinoic acid (RA)—have been shown to be involved in the early entry into or the repression of meiosis in mouse. Fgf9, genetically located downstream of Sry, constitutes a major component of positive feedback for establishing male determination [15]. This factor directly acts on germ cells to repress promotion of meiosis through Fgf2 receptor and Nanos2. RA is recognized as a factor for promotion of meiosis since disruption of RA signaling in gonads represses meiotic process. The two independent factors mutually act to regulate meiosis [16, 17]. Given that an early entry into meiosis...
associated with the mechanism of female determination in germ cells, these factors can be categorized as factors affecting sexual fate decision of germ cells.

Studies using the teleost fish provided further insights into the role of germ cell type for the sexual fate decision. Regardless of donor trout sex, germ cells that were transplanted into larva assumed the sexual fate of the recipient gonad [18, 19]. We also transplanted GFP-labelled germ cells isolated from adult testis into the coelomic cavity of larva and found that GFP-germ cells developed into oocytes (unpublished results). These results suggest that adult ovaries and testes contain sexually indifferent or unfixed germline stem cells. In fact, as described below, a clonal analysis demonstrated the presence of germline stem cells in the medaka ovary [20]. The germline stem cells in the medaka ovary continuously generate oocytes throughout the reproductive term, indicating the presence of germ cells with the ability to self-renew to maintain the stem cell population and to produce cells that can differentiate into eggs. The germ cells with these characters are developmentally uncommitted to gametogenesis and are likely to be sexually indeterminate (e.g., [21, 22]).

**Early gametogenesis – from germline stem cells to entry of meiosis**

Germ cells residing in the primordial gonad are often referred to as gonocytes or gonial cells. As primordial germ cells (PGCs) enter the primordial gonad, these cells acquire the capacity as gonial cells to develop into either eggs or sperms. Gonial cells in the mature testis were shown to include the germline stem cell population that is essential for continuous sperm production [23]. Several non-mammalian vertebrates, especially those that produce numerous eggs, are shown to possess germline stem cells or germ cells with stem cell markers [24]. In these vertebrates, gametogenesis from germline stem cell progenitors exhibits a conserved pattern of cellular division (Fig. 1).

In many organisms, progenitor cells undergo successive and synchronous mitoses, followed by entry into the first events of meiosis, the meiotic prophase I [25–28]. These successive mitoses are characterized by an incomplete cytokinesis, so that daughter cells remain connected through a cytoplasmic bridge. The interconnected germ cells are packaged as a cyst and surrounded by somatic cells. Following several rounds of mitosis, the cells enter meiosis to become eggs in the ovary or sperms in the testis [28, 29]. Interestingly, the number of successive division rounds differs between oogenesis and spermatogenesis in medaka [20, 30]. This suggests that gametogenesis-committed germ cells are sexually determined by the end of the cystic division stage.

Unlike the vertebrates mentioned above, mouse exhibits a different pattern of oogenesis in that the ovary does not retain typical germline stem cells [24] (compare Fig. 2A and B). The PGCs in mouse develop into gonocytes that are competent for gametogenesis [31]. However, this developmental status appears to be transient in female because all germ cells will eventually enter oogenesis during the fetal period. After the commitment into oocyte development, the pattern of germ cell division proceeds in a similar way as seen in non-mammalian vertebrates: the cystic division generates aggregates of germ cells that are partially interconnected to each other via intercellular bridges, and the germ cell aggregates subsequently break apart to form primordial follicles. This occurs only during a fetal period and, therefore, cystic germ cells are also absent in the adult ovary [25, 32, 33]. On the contrary, in mouse testis, gonocytes develop into spermatogonial cell populations including germline stem cells, which maintain the homeostasis of spermatogenesis [23].

The presence of germline stem cells only in male mice is atypical for vertebrates. Actually some mammals have been suggested to possess germ cells that undergo mitosis in the adult ovary [34]. The teleost and amphibian ovaries were shown to possess germline stem cells and/or mitotic germ cells [35]. Germline stem cells may exist in a prototype for the cellular gametogenesis in adult gonads.

**Expected timing of sexual fate decision of germ cells**

As described above, germline stem cells are very likely sexually indifferent or unixed even in adult gonads in the teleost while the typical germline stem cells are present only in male mouse but not in female mouse. Then the question...
Figure 2. The sexual fate decision of germ cells and the status of germline stem cells. The mechanism of sexual fate decision in germ cells may be linked to both the oogenesis process during the development and the germline stem cell status in the mature ovary. The loss of germline stem cells may allow for the neofunctionalization of sox9/amh, which contributes to the masculinization of the gonad in mouse. A: During the ovarian and testicular development of medaka, sexually indifferent and/or unfixed germline stem cells are established. The testis and the ovary determine the sexual fate of the progeny of mitotically quiescent germline stem cells. Downregulation of foxl3/Foxl3 is critical for the germ cell commitment to spermatogenesis. B: During mouse ovarian development, all germ cells enter oogenesis, which may be mediated by the transient status of the gonocytes. Adult ovaries preserve germ cells as follicles and do not possess the typical germline stem cells. Thus, the function of foxl3/Foxl3 may be dispensable in mouse. Unlike in medaka, germline stem cells in mouse testis might be committed to male.

arises regarding the timing of sexual fate decision of germ cells.

In medaka, prior to the pachytene stage of meiosis, female and male germline stem cells, and mitotically dividing cystic germ cells are morphologically indistinguishable. However, female germ cells are getting larger than male germ cells by the pachytene stage. This suggests that the fate decision to eggs or sperms is made prior to this stage (Fig. 1) and occurs in the period from the timing of commitment of germline stem cells to gametogenesis and the early pachytene stage.

In mouse, sexually distinct germ cell characters were demonstrated using several meiosis mutants [36]. During meiosis, checkpoints at early meiotic prophase I, such as the double strand break and the synaptonemal complex (SC) formation, interrupt the progression of meiosis. The stage of interruption differs in female and male germ cells as early as the timing of zygote. These observations suggest that germ cells possess sexually distinct characters before they enter zygote stage.

These observations suggest that the sexual fate decision is timed similarly in both mouse and medaka (after commitment of gonocytes/germline stem cells but before early stage of meiosis). An important point here in mouse studies is that the entry of meiosis during a fetal period has been regarded as a sign of female germ cell differentiation because male germ cells do not enter meiosis until a neonatal period. Therefore, the studies trying to address sexual fate decision have also been centered on the meiosis that occurs during female ovary development in the fetus.

Factors regulating early gametogenesis in the mouse

In this context, several germ cell-specific factors have been identified. One important factor is stra8. stra8 is an essential gene upregulated in germ cells responding to retinoic acid (RA) that is an exogenous factor promoting meiosis. The repression of meiosis in male fetus is shown to correlate with down-regulation of stra8 by male-specific factor of fgf9 [37]. Nanos2 is another factor involving the repression of meiosis in germ cells. Dysfunctional nanos2 in germ cells causes the precocious expression of meiotic genes during testicular development [38]. Both factors appear to prevent the precocious entry of male germ cells into meiosis. The polycomb repressive complex 1 (PRC1) may also contribute to the distinct sexual state of germ cells because premature expression of Stra8 is only observed in female germ cells of mutant gonads [39]. These mechanisms are consistent with the expected timing of the sexual fate decision.

It is important to note that these studies are based on the assumption that an event of the early meiosis and an event of feminization are nearly equivalent in germ cells. Nonetheless, an analysis of stra8 mutant seems to speak against this assumption. In the stra8 mutant, a very small number of germ cells can develop into oocyte-like cells without undergoing the meiosis process. The mutant oocyte-like cells have the capacity to be fertilized in vitro [40]. This analysis suggested that a yet to be identified molecule intrinsically participates in the sexual decision of the germ cells toward female (oogenesis), but not in the promotion of meiosis that occurs in the ovary. Thus, early meiotic entry during ovarian development may be linked to the mechanism of feminality in germ cells (therefore it can be used to indicate femaleness), but may not be equivalent to the sexual fate decision of germ cells to female. It would be possible that a
change of epigenetic status in male germ cells can be regarded as a process of stem cell establishment but not maleness of germ cells [38, 39].

**Discovery of foxl3 as a switch gene for sexual fate decision of germ cells**

The mechanism of sexual fate decision may be present and act in a cell-autonomous manner in germ cells. This event may take place between the germ-line stem cell stage and the pachytene stage. As an alternative mechanism, the sexual fate decision is not innate in the germ cells. In this scenario, the sexual fate decision of germ cells is mechanistically identical to the germ cell development toward eggs or sperms. Germ cells are unable to reach the end of their sexual path without receiving an instructive signal(s) from the surrounding somatic cells. In this case, oogenesis and spermatogenesis are controlled by the somatic cells in a stepwise fashion.

A recent finding on foxl3 function demonstrated that the germ cells indeed possess an intrinsic mechanism [41]. Foxl3 contains a fork-head domain and is expressed in germ cells in medaka. During a very early stage of the gonadal formation, foxl3/Foxl3 is transiently expressed in both female and male germ cells as early as the onset of sexual differentiation of the gonads (stage 35). However, foxl3/Foxl3 expression diminishes in the male, but is maintained in a subpopulation of stem type germ cells (type I germ cells) and in all cystic-type germ cells (type II germ cells) in the female (Fig. 1). Interestingly, mitotically quiescent stem type germ cells do not express Foxl3 in females, indicating that the commitment to gametogenesis is associated with the activation of foxl3/Foxl3 expression. As early events of meiosis (meiotic prophase I) take place in the germ cells, foxl3/Foxl3 expression diminishes in the female germ cells. This expression pattern is also consistent with the expected timing of the sexual fate decision described above.

The loss of foxl3 expression leads to a remarkable phenotype in the female. Female homozygous foxl3 mutants develop a typical ovarian structure and express ovary-specific genes. Normal oocytes develop within the germinal epithelium layer, termed the cortical zone, where germ cell stem cells are maintained, and oogenesis proceeds until the germ cells reach the early diplotene stage [20, 41]. Germ cells with a defective foxl3 gene develop sperms in the germinal epithelium of the developing ovary at the larval stage. As a result, the germinal epithelium expands with numerous sperms. The sperm cannot go out from the germinal epithelium because the mutant ovary does not develop a ductal system, such as an efferent duct in testes. But artificial insemination has shown that the isolated sperm from the mutant ovary are fertile. In turn, the fertilized eggs develop to term, sexually maturing as adult females with fertile eggs, producing off-spring. This phenotype clearly demonstrates the presence of an intrinsic mechanism for the sexual fate decision in germ cells.

**Duration and mechanism of the sexual fate decision in germ cells**

The mutant phenotype described above provides insights into the duration and mechanism of the sexual fate decision of germ cells. Here, it is noteworthy to mention that foxl3/Foxl3 expression is not detected in mitotically quiescent germ cells. It is well established that stem cell populations are not homogeneous, but instead are comprised of at least two populations – one that is mostly quiescent, and one that is mitotically active [20]. As the mitotically quiescent germ cells during medaka embryonic period display no gene expression of gametogenesis and meiosis and a large cell size with less prominent DAPI (4′,6-diamidino-2-phenylindole) staining, these quiescent cells are likely the stem of stem cells or are the gonocytes that are developing into germ-line stem cells. The foxl3/Foxl3 expression is initiated in some populations of mitotically active germ line stem cell type. Interestingly, the adult testis and ovary of medaka display a mosaic nanos2 (a hallmark of germ line stem cells) expression pattern in the type A spermatogonia and oogonia, including the germline stem cells, which reflect different grades of stemness characters within the gonial populations [24]. The medaka adult ovary also displays a mosaic foxl3/Foxl3 expression pattern in oogonia. Although, it is essential to examine if foxl3/Foxl3 and nanos2 are coexpressed in the gonial populations, it is possible that the loss of stemness character is related to initiation of foxl3/Foxl3 expression. The mosaic expression in gonial populations implies that the initiation of sexual fate decision may be linked to the establishment (in larva) and regulation (in adult) of stem cells.

The foxl3 mutant phenotype and expression of foxl3/Foxl3 also suggest the duration of the sexual fate decision during the course of gametogenesis. During a normal oogenic process, germ cells undergo to three to four rounds of successive cell divisions (maximum of five rounds), resulting in between 8 and 64 premeiotic germ cells within a single cyst. Furthermore, more than nine rounds of cystic division take place successively and clonally to produce a minimum of 512 spermatocytes in wild-type male [20, 30]. However, germ cells in the mutant female divide in a fashion similar to that observed in wild-type testis. Differences in the number of cystic divisions that occur between sexes suggests that the sexual fate decision process continues during the early stage of cystic division, which is earlier than the pachytene stage predicted solely by morphology.

Because the female foxl3 mutant exhibits mature sperm production in the developing ovary, this mutant phenotype clearly demonstrates that one major mechanism of the foxl3/Foxl3 sexual switch is to repress the initiation of spermatogenesis. Because of loss of the foxl3/Foxl3 function, derepression of spermatogenesis is initiated in the mutant around the time of hatching, which coincides with the timing of oocyte production in wild-type female. In contrast, germ cells in male foxl3/mutant initiate spermatogenesis earlier than those in wild-type male [41].

**Mature gonads are the organs that determine the sex of germ cells**

The finding that foxl3/Foxl3 expression initially occurs in some germ line stem cell populations supports the idea that
the sexual fate decision is initiated at the very beginning of the gametogenesis commitment in mitotically active germline stem cells. This finding led to an unconventional notion of the existence of a germ cell population, the sex of which is determined long after that of the organism (somatic cells) in medaka. In this case, mature ovaries and testes are not merely reproductive organs that regulate the process of gametogenesis (timing and quantity), but they also act to guide the sexual fate of germ cells by directing the expression of foxl3/Foxl3. To my knowledge, the view on adult testis and ovary as germ cell sex-determining organs has not been sufficiently addressed at the molecular level. However, the adult mouse ovary is different from that of medaka in that the mechanism of sexual fate decision of germ cells is dispensable since all germ cells are present as follicles and not as typical germline stem cells that are sexually indifferent and/or flexible (Fig. 2).

Rapidly evolving foxl3

This view may be associated with a failure to detect the foxl3 gene in the mammalian genomes examined thus far. However, the foxl3 gene is present in other vertebrates from teleost to marsupials [42, 43]. Thus, the evolutionary conservation of the foxl3 gene among other vertebrates may be linked to the presence of sex determining mechanism in the adult ovary. The absence of initial germ cells that trigger foxl3/Foxl3 expression in the mammalian adult ovary is in agreement with the fact that the mammalian adult ovary does not possess sexually indifferent or unfixed germ cells.

It is noteworthy to mention that the foxl3 gene shows a more rapid amino acid substitution rate than foxl2, foxl2 and foxl3 share the closest neighboring node in the phylogenetic tree. A duplication is thought to have occurred before teleosts diverged [42, 43]. In mammals, foxl2/Foxl2 is known to be essential for development and maintenance of female somatic cells, foxl2/Foxl2 in both mammals and teleost is expressed in somatic cells of only female gonads but not of male gonads, suggesting that the foxl2 of teleost and mammals share a similar role [44–49]. In contrast to the conserved role of foxl2/Foxl2, the rapid amino acid substitution rate of foxl3 implies that its function changed at a relatively fast rate during vertebrate evolution. In particular, in bird and marsupial branches, a branch of foxl3 clades is extended long in the phylogenetic tree, suggesting the possibility that the function of foxl3/Foxl3 is changing. As evolution proceeds toward mammalian clades, foxl3/Foxl3 might have lost the function of sex determination in germ cells [42, 43]. It is likely that the changes in the mechanism of germ cell sexual fate decision are linked with those in foxl3. The development of a novel decision mechanism may have accompanied the loss of germline stem cells. Additionally, the fact that germline stem cells in the ovary are unnecessary/not required may have promoted novel sex determination mechanisms.

Neofunctionalization of other genes with loss of germline stem cells in mammals

Actually, the development of a novel mechanism may cause the striking change of sex determination mechanism of the somatic cells. Sox9 is a direct effector of the mammalian somatic sex determination gene, Sry. Sry is a mammalian-specific gene, while Sox9 is conserved among vertebrates [50]. However, our recent analysis indicated that sox9b, an orthologue of the mammalian Sox9 expressed in the supporting cells – is not directly involved in testicular differentiation. A study utilizing a medaka sox9b mutant demonstrated that the function of sox9b is more related to the maintenance of germ cells [51]. The mutant also indicated that sox9b expression is more intense in the supporting cells surrounding the germline stem cell population than in those that surround the gametogenesis-committed germ cells [24]. The function of germ cell maintenance has also been reported in the mouse adult testis [52]. It is speculated that the loss of sox9b function in the germ cell maintenance, in addition to the loss of germline stem cells, allows for the neofunctionalization of the mammalian Sox9 as an effector of Sry.

A similar scenario may also apply to the function of the anti-Müllerian hormone (AMH/MIS). AMH/MIS is a phylogenetically ancient molecule that belongs to the bone morphogenetic protein (BMP) gene family and is regulated downstream of Sox9 during testicular development in mammals. AMH/MIS is essential for the regression of female reproductive organs, such as the upper vagina and oviduct. However, an analysis of the mutant of a type II receptor of AMH/MIS in medaka indicated AMH/MIS involvement in the regulation of the germline stem cell numbers, but not in the formation of reproductive organs [53, 54]. This led to a hypothesis that the mammalian AMH/MIS acquired its role in the regression of female reproductive organs because the establishment of germline stem cells is dispensable during mammalian ovarian formation.

The default sexual status of germ cells – the role of foxl3/Foxl3

The role of foxl3/Foxl3 has raised a question about the default sex of germ cells. As mentioned above, immediately following PGC residence in the gonadal primordium, foxl3/Foxl3 is detected in both female and male. Subsequently, foxl3/Foxl3 expression is repressed specifically in male. This pattern of expression, in conjunction with the mutant phenotype, led us to conclude that the function of foxl3/Foxl3 is to repress spermatogenesis and that the repression of foxl3/Foxl3 results from some act by masculinized somatic cells. In other words, the gonocytes expressing foxl3/Foxl3 are likely in the default status.

Then, one might argue that foxl3/Foxl3 has a positive role in oogenesis because foxl3/Foxl3 is continuously expressed in the female germ cells. Interestingly, considering the absence of a foxl3/Foxl3 orthologue in mammals, foxl3/Foxl3 might not be required for the entry into oogenesis. The phenotype of the foxl3 mutant at the adult stage may also support this notion, because few fertile oocytes appear
in a later mature stage of the sperm filled-mutant ovary [41]. This suggests that oocytes can develop in the absence of foxl3/Foxl3. These facts collectively imply that not becoming male germ cells leads to female germ cells where foxl3/Foxl3 may not contribute to becoming female germ cells. On the other hand, we do not yet know if not becoming female is equal to development to male germ cells. Forcing the expression of foxl3/Foxl3 in germ cells of the testis would elucidate the default sex of the germ cells.

Conclusions and outlook

Here, I have described the concept that foxl3 has changed rapidly during the course of evolution. Nonetheless, I postulate the conservation of foxl3, because it is more conserved than the genes that are involved in the sexual fate decision of somatic cells (genes that are conventionally referred to as “sex determination genes”). The sex determination genes tend to change more rapidly, and are typically not conserved, even within a single class [55, 56]. However, the foxl3 gene is found in the teleost, amphibian, reptile, bird, and marsupial genomes. Mammals are the only vertebrates that do not possess foxl3 gene, which is consistent with mammals also not having typical germ-line stem cells in the ovary. Further investigation of the function of foxl3/Foxl3 in other classes of organism, and the presence of germline stem cells (especially in birds because germline stem cells are not yet identified and foxl3 gene changes a lot compared to that in teleost) will provide a more comprehensive picture of how sexual fate decision is orchestrated in somatic as well as germ cells, and how diverse mechanisms are employed in different species.

Acknowledgments

I acknowledge all the current and previous members in my lab, especially Dr. T. Nishimura for analysis of foxl3 and discussion.

The author has declared no conflict of interest.

References

1. Marshall Graves JA. 2013. How to evolve new vertebrates sex determining gene. Dev Dyn 242: 384–99.
2. Sinclair AH, Berta P, Mark S, Hawkins JR, et al. 1990. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 346: 249–52.
3. Koopman P, Gubbay J, Vivian N, Goodfellow P, et al. 1991. Male development of chromosomally male mice transgenic for Sry. Nature 351: 117–9.
4. Larney C, Bailey TL, Koopman P. 2014. Switching from oogenesis to spermato- genesis in the testis would elucidate the default sex of the germ cells.
5. Matsuoka N, Nagahama Y, Shinomiya A, Sato T, et al. 2002. DMY is a Y-specific DM-mouse gene required for male development in the medaka fish. Nature 417: 559–63.
6. Nanada I, Kondo M, Hormung U, Asakawa S, et al. 2002. A duplicated copy of DMR17 in the sex-determining region of the ZW-type sex determining system in the medaka, Oryzias latipes. Proc Natl Acad Sci USA 99: 11778–83.
7. Smith CA, Roeszler KN, Ohnoes T, Cummins DM, et al. 2009. The avian Z-linked gene DMR17 is required for male sex determination in the chicken. Nature 461: 267–71.
8. Yoshimoto S, Ikeda N, Izutsu Y, Shiba T, et al. 2010. Opposite roles of DMR17 and its W-linked parologue, DM-W, in sexual dimorphism of Xenopus laevis: implication of a Z/W-type sex determining system. Development 137: 2519–26.
9. Hattori RS, Murai Y, Oura M, Masuda S, et al. 2012. A Y-linked missense SNP in Amhr2 is associated with sex determination in the tiger pufferfish, Takifugu rubripes (fugu). PLoS Genet 8: e1002798.
10. Yano A, Guyomard R, Nicol B, Jouanno E, et al. 2014. Co-option of Sox3 as the male-determining factor on the Y chromosome in the fish, Oryzias latipes, during the winter months. J Exp Zool 245: 71–77.
11. Gill ME, Hu YC, Lin Y, Page DC. 2011. Licensing of gametogenesis, dependent on RNA binding protein DAZL, as a gateway to sexual differentiation of feline germ cells. Proc Natl Acad Sci USA 108: 7443–8.
12. Mork L, Tang H, Batchvarov I, Capell B. 2012. Mouse germ cell clusters form by aggregation as well as clonal divisions. Mech Dev 128: 591–6.
13. Lei L, Spradling AC. 2013. Mouse primordial germ cells produce cysts that partially fragment prior to meiosis. Development 140: 2075–81.
14. Anand Kumar TC. 1988. Oogenesis in Loises: Loris tardigradus lydekienanus and Nycticebus couang. Proc Roy Soc B 169: 167–76.
15. Shiba N, Hamaguchi S. 1997. Evidence for the sexual bipolarity of spermatogonia in the fish, Oryzias latipes during the winter months. J Exp Zool 245: 71–77.
16. Gill ME, Hu YC, Lin Y, Page DC. 2011. Licensing of gametogenesis, dependent on RNA binding protein DAZL, as a gateway to sexual differentiation of feline germ cells. Proc Natl Acad Sci USA 108: 7443–8.
17. Bowles J, Feng CW, Spiller C, Davidson TL, et al. 2010. FGFR9 suppresses meiosis and promotes male germ cell fate in mice. Dev Cell 19: 440–9.
18. Barrios F, Filipponi D, Pellegrini M, Paronnett MP, et al. 2010. Opposing effects of retinoic acid and FGFR9 on Nanos2 expression and meiotic entry of mouse germ cells. J Cell Sci 123: 871–80.
44. von Schalburg KR, Gowen BE, Rondeau EB, Johnson NW, et al. 2013. Sex-specific expression, synthesis and localization of aromatase regulators in one-year-old Atlantic salmon ovaries and testis. Comp Biochem Physiol B Biochem Mol Biol 164: 236–46.

45. Zhou L, Charkraborty T, Zhou Q, Mohaptra S, et al. 2016. Rspon1-activated signalling molecules are sufficient to induce ovarian differentiation in XY medaka (Oryzias latipes). Sci Rep 6: 19543.

46. Lin YT, Capel B. 2015. Cell fate commitment during mammalian sex determination. Curr Opin Genet Dev 32: 144–52.

47. Heule C, Goppert C, Saltzburger W, Bohne A. 2014. Genetics and timing of sex determination in the East African cichlid fish Astatotilapia burtoni. BMC Genet 15: 140.

48. Herpin A, Adolfo MC, Nicol B, Hinzmann M, et al. 2013. Divergent expression regulation of gonad development genes in medaka shows incomplete conservation of the downstream regulatory network of vertebrate sex determination. Mol Biol Evol 30: 2328–46.

49. Nakamura S, Watakabe I, Nishimura T, Toyoda A, et al. 2012. Analysis of medaka sox9 orthologue reveals a conserved role in germ cell maintenance. PLoS ONE 7: e29982.

50. Barrionuevo F, Georg I, Scherthan H, Lecureuil C, et al. 2009. Testis cord differentiation after the sex determination stage is independent of Sox9 but fails in the combined absence of Sox9 and Sox8. Dev Biol 327: 301–12.

51. Morinaga C, Saito D, Nakamura S, Sasaki T, et al. 2007. The hotel mutation of medaka in the anti-Müllerian hormone receptor causes the dysregulation of germ cell and sexual development. Proc Natl Acad Sci USA 104: 9691–6.

52. Nakamura S, Watakabe I, Nishimura T, Picard JY, et al. 2012. Hyperproliferation of mitotically active germ cells due to defective anti-Müllerian hormone signaling mediates sex reversal in medaka. Development 139: 2283–7.

53. Whittfield LS, Lovell-Badge R, Goodfellow PN. 1993. Rapid sequence evolution of the mammalian sex-determining gene SRY. Nature 364: 713–5.

54. Kikuchi K, Hamaguchi S. 2013. Novel-sex-determining genes in fish and sex chromosome evolution. Dev Dyn 242: 339–53.