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Germ Cell Sex Determination: A Collaboration between Soma and Germline

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Abstract

Sex determination is regulated very differently in the soma vs. the germline, yet both processes are critical for the creation of the male and female gametes. In general, the soma plays an essential role in regulating sexual identity of the germline. However, in some species, such as *Drosophila* and mouse, the sex chromosome constitution of the germ cells makes an autonomous contribution to germline sexual development. Here we review how the soma and germline cooperate to determine germline sexual identity for some important model systems, the fly, the worm and the mouse, and discuss some of the implications of “dual control” (soma plus germline) as compared to species where germline sex is dictated only by the surrounding soma.

The differences between the sexes, what makes us male or female, has been a topic that has captured the imagination of scientists for many years. Most emphasis has been placed on the mechanisms that control somatic sex, as this creates the dramatic differences in morphology and behavior that distinguish the sexes. However, from mother nature’s perspective, the point of sexual reproduction is to get the male and female gametes together to ensure propagation of the species. Thus, sex determination in the germ cells that give rise to the gametes is of paramount importance.

In most animals, the pathways that govern intrinsic sexual identity in the germline are distinct from those in the soma. The soma often plays an important role in determining the sex of the germ cells, consistent with the idea that the primary role of the soma is to nurture and ensure the success of the germ cells. In some species, the soma is sufficient to determine the sex of the germ cells, and there is no autonomous sex determination mechanism in the germ cells themselves. In these animals, such as the housefly [1], frog [2], and some fish [3–5], when germ cells are placed into a somatic environment of a different sex through transplantation or reversing the sex of the soma, they give rise to sperm or egg depending on the sex of their host and independent of their own sex chromosome constitution. However, in other species, the sex chromosome constitution also plays an autonomous role in the germ cells. In these animals, such as the fruitfly and the mouse, the “sex” of the soma must match the “sex” of the germ cells for proper gametogenesis to take place. There are several ways in which the sex chromosome constitution might matter to the germ cells, including genes required for spermatogenesis on the Y chromosome and differences in the way dosage

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compensation in the germline or sex chromosome pairing during meiosis might occur between the sexes. However, there is also clear evidence that the sex chromosomes can influence the sexual identity of the germline in an autonomous manner, in addition to any signals these germ cells receive from the soma.

Here we describe how signals from the somatic environment combine with germ cell autonomous mechanisms to control germline sex determination in some well-characterized model systems. Sex determination in the germline and soma of each of these models has been recently reviewed [6–11], and so we will focus on comparing these different systems. We also discuss how such dual control over germline sex might influence mechanisms controlling sexual identity in general.

Drosophila

In *Drosophila melanogaster*, it is clear that germline sex determination involves both germline autonomous cues and signals from the surrounding somatic gonad. Transplant studies have shown that XX germ cells do not form sperm in a male soma and XY germ cells do not form oocytes in a female soma [12,13]. Thus, the sex of the germline must match that of the soma for proper germ cell development to occur. Similarly, genetic techniques have allowed sex-reversal in the somatic cells but not the germline, resulting in “XX pseudomales” and “XY pseudofemales”. In the resulting gonads, some of the germ cells may appear to initiate spermatogenesis while others appear to initiate oogenesis [14,15] though, universally, the germline in these gonads degenerates resulting in sterility. Work in this field has begun to characterize the separate somatic and germline pathways that control germline sexual identity.

Somatic Control Over Germline Sex

In *Drosophila*, somatic cells determine their sex by counting the number of X chromosomes, with XX being female and XY male (reviewed in [6]). The Y chromosome does not influence sexual identity, but does contain some genes required for spermatogenesis. The presence of two X chromosome in females initiates the expression of Sex lethal (SXL), a key player in two sex-specific pathways: SXL blocks dosage compensation in females by preventing expression of Male-specific lethal 2 (MSL2) [16] and regulates somatic sex by initiating an alternative-splicing cascade acting through Transformer (TRA) and Transformer 2 (TRA2). This cascade regulates sex-specific splicing of the mRNAs expressing the transcription factors Doublesex (DSX) and Fruitless (FRU) (Fig. 1). DSX is the key factor regulating sexual dimorphism of the somatic gonad [17,18] and is expressed in a subset of somatic gonadal cells [19–21]. DSX can also control sexually dimorphic development of somatic gonadal cells that do not express DSX via non-autonomous cell-cell signaling [22] and is responsible for how the somatic gonad influences sex determination in the germline [23].

Sexual identity in the germline can be detected soon after the gonad first forms in the embryo [24–26]. Both male and female-specific gene expression can be observed in the germline at this time, and the soma plays a dominant role in determining which pattern of expression is observed: XX germ cells in a male soma exhibit a male-like pattern while XY germ cells in a female soma exhibit a female-like pattern [24,26]. Later in development, however, both XX and XY germ cells in a soma of the wrong sex exhibit a confused sexual identity [15,26,27]. Thus, the somatic signals are not sufficient to maintain the sex of adult germ cells. The signals by which the soma influences germline sex have only just begun to be identified. The male somatic gonad activates the JAK/STAT pathway in the germline, and this has a masculinizing effect on the germ cells [28]. The female soma also plays a

clear role in regulating germline gene expression [24,29], but the mechanism by which this occurs remains undiscovered.

Germine Sexual Identity

In addition to somatic signals, the sex chromosome constitution of the germ cells is important for germline sex determination. Though the germ cells rely on the number of X chromosomes to determine autonomous sexual identity, the mechanism behind this is completely different than for the soma (reviewed in [7,30]). The key genes acting in somatic sex determination, *tra* and *dsx*, are not required in the germ cells [31,32]. In addition, while *Sxl* is required to promote female germ cell identity [33], the way in which it is activated in female germ cells, and its downstream targets, are different than for somatic sex determination [31,34,35]. Further, expression of SXL in the male germline does not lead to feminization as it does in the soma [36], indicating that its role in establishing germline sex is far less pivotal than in somatic sex determination.

Mutations in *Sxl* germline function lead to germline overproliferation (ovarian tumors), similar to when an XY germ cell is transplanted into a female soma [13,33]. Mutations in the genes *ovo* and *ovarian tumor (otu)* have a similar phenotype [37,38], and evidence indicates that these genes cooperate with *Sxl* in promoting female germ cell identity. *ovo*, which encodes a transcription factor OVO-B [39–41], is a regulator of *otu* [42], and both appear to act upstream of *Sxl* in the germline [43,44]. *otu* belongs to a superfamily of deubiquitinating enzymes [45] and may therefore alter protein covalent modifications by removing ubiquitin or SUMO, a prevalent modification in germ cells [46]. *ovo* promoter activity is higher in XX germ cells than in XY or XO germ cells, suggesting that *ovo* expression is autonomously regulated by X chromosome number; however, *ovo* expression in XY germ cells can also be upregulated by a female somatic environment [44]. Similarly, *otu* expression is regulated by two mechanisms in the XX germline, one is OVO-B dependent and the other is dependent on female somatic inductive signals [14]. By providing information through these genetic pathways, germline sexual identity can be influenced by the somatic environment but is also based on sex chromosome composition.

C. elegans

In *C. elegans*, somatic sexual identity is also regulated by X chromosome number: XO individuals are male, whereas XX worms are hermaphrodites that are somatically female but produce sperm briefly before switching to egg production. This poses unique challenges in establishing germline sexual programs; hermaphrodites need to accommodate both male and female germline development as well as orchestrating the switch from spermatogenesis to oogenesis. Over the last few decades, a large body of work has elucidated the genetic pathway that drives male vs. female germline development and these findings have been summarized in several comprehensive reviews [8,9,47]. Importantly, unlike in *Drosophila* and mammals, germ cells in *C. elegans* respond primarily to the somatic environment to determine their sex, and their own sex chromosome constitution does not appear to play an autonomous role.

Somatic input into germline sex

In worms, X chromosome number (X:A ratio) is “read out” by XOL-1 (XO Lethal) which is repressed in hermaphrodites and activated in males (Fig 1). XOL-1 expression leads to repression of SDC-1-3 (Sex determination and Dosage Compensation defective 1–3) and allows for expression of HER-1 (Hermaphrodization of XO animals) [8]. HER-1 is a small secreted peptide produced only in males, and it acts system-wide to drive male development. It inactivates its cognate receptor Transformer 2 (TRA-2), which in turn allows the

Feminization proteins (FEM-1, FEM-2, and FEM-3) to target the transcription factor Transformer-1 (TRA-1) for degradation and promote male identity. (It should be noted that TRA-1 and TRA-2 in *C. elegans* are not homologues to *Drosophila* TRA proteins.) Without HER-1, TRA-2 remains active to promote hermaphrodite identity. Mosaic analyses demonstrated that HER-1 expression in the soma promotes male identity in the germline, and is sufficient to masculinize either XO or XX germ cells [48,49]. Thus, the sex chromosome constitution of the germline itself is not critical and the non-autonomous signal, HER-1, that allows somatic cells to “agree” on their sex, is also the mechanism by which the soma controls germline sex determination. However, beyond the core sex determination components, there are important differences between the soma and germline. In the germline, the FEM genes have a second role in promoting spermatogenesis independent of regulating TRA-1 [50,51]. In addition, there are germline-specific targets of TRA-1 that include spermatogenesis genes FOG-1 and FOG-3 (Feminization Of Germline, [52,53]).

Regulation of the sperm-oocyte switch

While the initial germline sex determination decision is controlled by the soma, additional regulation of this decision still occurs in the germline, although it does not appear to be regulated by the germline sex chromosome constitution. Most species of *Caenorhabditis* are not hermaphroditic but are male/female, and require only a single germline sex determination decision. In *C. elegans*, the “females” have evolved a mechanism for temporarily blocking female identity in the germline, allowing for some sperm to be produced [54]. Indeed, a male/female *Caenorhabditis* species, *C. remanei*, can be transformed into a hermaphroditic species by suppressing TRA-2 and regulating sperm activation [55].

In female germ cells, the absence of HER-1 allows TRA-2 to be active and promote a female identity. For hermaphrodites to make sperm, the TRA-2 pathway must be transiently inhibited. This is achieved through translational repression of TRA-2 by FOG-2 and Germline Defective-1 [56–58]. In late larvae, a change in germline sex needs to be implemented to switch to oogenesis. FEM-3 Binding Factors (FBF-1 and -2) work together with Nanos-3 to repress the translation of FEM-3 mRNA to allow oogenesis [59–62]. In addition, Masculinization of the Germline (MOG) 1–6 are required for FEM-3 repression [63–65], but the detailed mechanisms are yet to be elucidated. The temporal control of this switch is also not understood. Two other factors, Y14 and MAG-1 (homolog of *Drosophila* Mago Nashi), also contribute to the transition, possibly post-translationally [66,67]. Thus, while germline sex is controlled by the soma and is independent of the germ cells’ sex chromosome constitution, an elegant control mechanism downstream of the initial sex decision is present in the germline of hermaphrodites to control the sperm-oocyte switch. Further, additional input from the soma may also influence the sperm to oocyte switch [68,69].

Mouse

Studies in mice indicate that, as in *Drosophila* and contrary to *C. elegans*, a combination of germline sex chromosome constitution and somatic environment determine the sexual identity of the mammalian germline (reviewed in [10]). Early evidence supported the role of the soma in controlling germline sex. Germ cells in a female somatic environment enter meiosis at a time typical of female germ cells regardless of whether they are XX or XY [70]. In a testis environment, XX and XY germ cells both behave typical of male germ cells, and undergo cell cycle arrest in the embryonic gonad rather than entering meiosis [71,72]. On the other hand, XX germ cells in a testis, as well as XY germ cells in an ovary, do not complete gametogenesis and degenerate prior to birth [70,71,73]. Thus, aspects of germline

sex-specific development are clearly determined by the germline sex chromosome constitution. The evidence for both autonomous (based on germline sex chromosomes) and non-autonomous (based on somatic environment) germline sexual identity will be explored.

Somatic influence over germline sexual identity

In the mouse soma, the presence of the Y chromosome and expression of the Sex-determining region on the Y (*Sry*) gene produces a male phenotype (reviewed in [11]). Failure to express *SRY* in XY individuals results in a female somatic phenotype, and expression of *SRY* in XX individuals is sufficient to masculinize the soma [74,75]. The *SRY* HMG-box transcription factor activates expression of the related factor *SOX9* [76] to promote male Sertoli cell formation and activate testis development [77](Fig. 1). *SOX9* also induces expression of the secreted fibroblast growth factor, *FGF9*, which reinforces male development and represses female development [78]. In contrast, in the female, the transcription factor *FOXL2* keeps *Sox9* off and leads to elevated *WNT4* expression that reinforces female gonad development and represses the male pathway [79–81].

How might the sex of the soma, then, influence germline sexual identity? *FGF9* acts as an extracellular signal while reinforcing male somatic identity, making it an ideal factor to convey a male somatic environment to adjacent germ cells [80]. In fact, direct *FGF9* signaling to germ cells is required for germline (XX or XY) survival in a male soma [82], demonstrating the capacity for germline to receive *FGF9* signals. Thus far, other candidate secreted molecules, such as *Wnt4*, appear to be involved primarily in somatic sexual identity [83].

The somatic gonad also regulates germline sexual identity through sex-specific modulation of retinoic acid (RA) signaling. RA is detected in the urogenital region of both males and females, but at much higher levels in the female gonad than the male [84]. This is due to sex-specific expression of the RA-degrading enzyme *CYP26B1* in the male somatic gonad [84,85]. Female germ cells respond to RA by upregulating the factor *STRA8* (stimulated by retinoic acid 8) that promotes meiotic entry [86,87]. Thus, higher levels of RA in the female gonad likely account for why female germ cells enter meiosis soon after gonad formation, while male germ cells do not, a sex-specific feature of mouse germ cell development that is commonly assayed in the study of germline sex determination.

However, while the timing of meiosis is a sex-specific trait, meiosis itself is not, and thus it is essential to study additional characteristics of germline sexual identity. The RNA-binding protein *NANOS2* is expressed specifically in male germ cells in mouse [88] and humans [89] and is essential for male germ cell development and survival [88]. *NANOS2* represses *STRA8* and meiosis, prevents translation of gene products involved in oogenesis [90], and is necessary and sufficient to promote aspects of male germ cell identity [91]. Male-specific expression of *NANOS2* results from activation by *FGF9* in male gonads and repression by RA in female gonads [91,92], making it key for interpreting sexual identity signals from the somatic gonad.

Germline autonomous sexual identity

A fundamental example of intrinsic differences between male and female germ cells can be seen in the inability for germ cells to differentiate properly when their sex chromosome constitution doesn't match that of the soma. Studies in XXY male mice show that they experience severe germline loss and most testis tubules are devoid of germline. The only foci of spermatogenesis observed are derived from germ cells that have randomly lost one X chromosome [72,93]. This cannot be explained simply by problems that the sex chromosomes may experience when pairing in meiosis, since premeiotic germline stem cells

and spermatogonia are also lost in these mice. Thus, though the Y chromosome is sufficient to masculinize somatic cells, two X chromosomes may confer female character to the germline, resulting in germline and soma with different sexual identities.

Additional evidence exists for the role of X chromosome number in influencing germline sexual development in mice. In sex-reversed (XX or XO male) mice, XX germ cells tend to behave as male, but are more likely to enter early meiosis (a female trait) than XY or XO germ cells [71]. Similarly, XO germ cells in a male somatic environment are able to progress further in spermatogenesis than XX germ cells [94,95]. More recently, molecular studies demonstrated that the sex-specific pattern of DNA methylation (imprinting) in the germline is also influenced by the number of X chromosomes in the germ cells, in addition to the sex of the surrounding soma [96,97]. Taken together, this evidence indicates that the sex chromosome constitution of the germ cells in mice influences sex-specific development of the germ cells, in addition to the signals that the germ cells receive from their somatic environment.

Implications

The mechanisms that determine sexual identity are diverse among different animal species. While there is evidence in the soma that the downstream pathways affected by these mechanisms may be more conserved [98], it is clear that the initial sex determination “switches” evolve rapidly. However, in some cases like mammals and fruit flies, sex determination switches appear more stable among related species while in other cases, such as frogs or fish, such switches appear to evolve more rapidly. There are even examples of single species utilizing multiple independent mechanisms for sex determination, such as in the housefly [99]. When there are independent mechanisms that determine sex in the soma and sex in the germ cells, this is likely to place an additional constraint on the evolution of sex determination mechanisms, since changes in either somatic sex or germline sex alone would be incompatible with fertility. Thus, one hypothesis is that sex determination mechanisms would be more stable in species with independent somatic and germline sex determination mechanisms, and there is some support for this idea in nature.

In addition to questions of evolution, our understanding of sex determination has profound implications for human development and disease. Many human syndromes lead to Disorders of Sexual Development (DSDs), which often cause defects in germ cell development and fertility. Such syndromes include genetic alterations of the sex determination pathway, such as SRY, which lead to XX males and XY females [100]. Other cases involve aneuploidy of the sex chromosomes, such as in XO females (Turner’s Syndrome) or XXY males (Klinefelter’s Syndrome). Almost invariably, such syndromes lead to severe defects in germ cell development. For example, in XXY males, the germ cells are commonly depleted before puberty [101], and in adults, most seminiferous tubules in the testis lack germ cells. When germ cells survive, they have an XY genotype due to loss of one X chromosome [102,103]. As in mice, the loss of XXY germ cells cannot be attributed solely to defects in meiosis, since germline stem cells and premeiotic spermatogonia are also lost [101]. Thus, a better understanding of how initial germline sex determination is regulated by both somatic signals and the germ cell’s own sex chromosome constitution is critical for understanding both normal and abnormal germline development.

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Fig 1.
Schematic diagram of how germ cells in *Drosophila*, *C. elegans*, and mice integrate intrinsic vs. somatic signals to decide their sexual identity. In *Drosophila* and mice, the germ cells combine intrinsic information with cues from the soma to decide their sex. In contrast, *C. elegans* germline sex depends on a non-autonomous signal from the soma. Blue and pink colors indicate factors and pathways that promote male and female development, respectively.