HHS Public Access

Author manuscript

Sex Dev. Author manuscript; available in PMC 2023 September 29.

Published in final edited form as:

Sex Dev. 2022 ; 16(5-6): 323–328. doi:10.1159/000521235.

The regulation of germline sex determination in *Drosophila* by *Sex lethal*

Lydia Grmai[*],

Cailtin Pozmanter[*],

Mark Van Doren

Department of Biology, Johns Hopkins University, Baltimore, MD.

Abstract

The establishment of male or female identity (sex determination) is essential for creating the anatomical, physiological, and behavioral differences between two sexes of the same species (sexual dimorphism). In many organisms, including mammals and *Drosophila*, sex is determined by inheritance of sex chromosomes while, in other animals, sex is determined by environmental factors. Arguably the most important consequence of sex determination is the production of healthy gametes necessary for reproduction: female oocytes and male spermatids. Generation of sperm and oocytes requires cooperation between two different cell types within the gonad: germ cells and somatic cells. Defects in sex determination in either the somatic gonad or germline lead to Disorders of Sexual Development and infertility. In *Drosophila*, the gene *Sex lethal (Sxl)* is the key determinant of sex in both the soma and the germline. However, how *Sxl* controls sex determination is much more well understood in the soma than the germline. This review will focus on *Sxl* in the germline: how it is activated specifically in female germ cells and how it regulates germline sex determination and sexual development.

Keywords

Sxl ; germline; sex determination; *Drosophila*

Sexual dimorphism in the Drosophila germline

In *Drosophila*, germ cells are established early in development and migrate through the embryo to join with somatic gonadal precursors to form the gonad (reviewed by [Jemc, 2011]).The earliest signs of sexual dimorphism in the gonad manifest in both germline and somatic cells and are apparent at the time of gonad coalescence in the embryo. In somatic cells, sex-specific isoforms of the key sex determining transcription factor, Doublesex

Corresponding author: Mark Van Doren, Department of Biology, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218, 410-516-4717, vandoren@jhu.edu.

Author Contributions:

The manuscript was researched, written and edited by LG, CP and MVD.

[*]These authors contributed equally to the work

Conflict of Interest Statement:

The authors have no conflicts of interest to declare.

(Dsx), are expressed in the somatic gonadal precursor cells [Hempel and Oliver, 2007]. Interestingly, homologs of *Drosophila* Dsx (the Dsx/Mab3 Related Transcription Factors or DMRTs) are now known to control sexual development of the somatic gonad in most or all animals where they have been studied, including mice and man [Zarkower and Murphy, 2021]. Sex-specific development of the somatic gonad in *Drosophila* begins at the time of initial gonad formation, and is apparent in both sex-specific gene expression and cell behavior [DeFalco et al., 2003; DeFalco et al., 2008].

Sex differences can also be detected in the germline at this stage. A greater number of germ cells incorporate into the male gonad than the female during gonad coalescence [Poirie et al., 1995]. In addition, sex-specific gene expression is apparent in the germline soon after the gonad forms [Staab et al., 1996; Casper and Van Doren, 2006]. Further, a male bias is observed in signaling activity via the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway in the germline [Wawersik et al., 2005]. This is regulated by the sex of the surrounding somatic cells, as only a male soma is capable of activating JAK/STAT signaling in the germline. Male-specific JAK/STAT activity is required for both proper male germline gene expression and cell behavior/proliferation [Wawersik et al., 2005]. Male-specific JAK/STAT activity soon becomes restricted to a subset of germ cells and is required for these cells to become germline stem cells (GSCs) [Sheng et al., 2009].

Arguably the most important place in the germline for sex determination is in the germline stem cells (GSCs), which must maintain the undifferentiated pool of cells that gives rise to the differentiating gametes. The timing of GSC formation is different between males and females, with male GSCs already present by the end of embryogenesis [Sheng et al., 2009], while female GSCs are established later during larval or early pupal development. This is largely thought to be regulated by the timing of GSC niche formation in the soma, which is controlled by the somatic sex determination gene *dsx* [Camara et al., 2019]. There are many interesting cell-cell signaling and cell biological differences in how male versus female GSCs are regulated and produce their differentiating daughter cells, and these differences have been extensively reviewed elsewhere [Fuller and Spradling, 2007; Yuan and Yamashita, 2010]. Lastly, the process of spermatogenesis and oogenesis are also very different, and how male versus female GSCs are able to produce daughter cells that enter such different developmental programs is a key aspect of germline sexual development about which little is known.

What controls these sex-specific differences in behavior and gene expression in the germline? In many animals, germ cells receive cues from the surrounding soma that instruct their sexual identity. In some animals, however, the sex chromosome genotype of the germline itself also plays a role in this process. In *Drosophila*, XY (normally male) germ cells fail to form proper oocytes when transplanted into a female somatic environment. Instead, they are often lost during development, and those remaining germ cells that populate the ovary form cysts of over-proliferating germ cells that fail to differentiate [Van Deusen, 1977; Schüpbach, 1985; Steinmann-Zwicky et al., 1989]. Similarly, XX germ cells transplanted into a male soma fail in spermatogenesis [Steinmann-Zwicky et al., 1989]. Thus, proper germline sexual identity requires autonomous information from the sex

chromosome genotype of the germ cells combined with proper signals from the surrounding soma. Interestingly, while mouse germline sex determination is commonly thought to be regulated strictly by signals from the soma, gametogenesis fails when the sex chromosome genotype of the germ cells does not match that of the surrounding soma, and increasing evidence indicates that the germline sex chromosome genotype is critical for mouse germ cell sexual development as well [Hamada et al., 2020].

As we will discuss, *Sxl* is the key sex determination factor in both the *Drosophila* germline and the soma. Sxl is an RNA-binding protein that acts in both RNA splicing and translational regulation and is necessary and sufficient for female sexual identity in somatic cells and in the germline. The role of Sxl specifically in the germline was first demonstrated by transplanting *Sxl*-mutant germ cells into normal female hosts, which yielded ovaries with germline tumors similar to those formed when XY germ cells are transplanted into a female host [Schüpbach, 1985]. Strikingly, *Sxl* is also sufficient to instruct female germline identity. If XY germ cells transplanted into a female host are forced to express Sxl, oogenesis is restored and fertile eggs are made [Hashiyama et al., 2011]. Thus, key questions regarding germline sex determination in *Drosophila* focus on how *Sxl* is activated specifically in the female germline, and which RNAs are being regulated to influence germline sexual development.

Activation of Sxl

In somatic cells, *Sxl* is activated according to the number of X chromosomes: cells with 2 or more X chromosomes initiate Sxl expression while cells with only a single X do not (Somatic sex determination has been extensively reviewed e.g. [Cline, 1993; Salz and Erickson, 2010] and we will only summarize this briefly). Because X chromosome dosage compensation equalizes gene expression from the X chromosome in 1X flies to equal that of 2X flies, X chromosomes can only be “counted” early in development before the onset of dosage compensation. The key step in Sxl expression is activation of the “early” or “establishment” promoter *Pe*. The transcript from *Pe* can produce a functional Sxl protein using the default RNA splicing machinery, providing a pulse of early Sxl protein. Shortly after, *Pe* is turned off in females and the “maintenance” promoter *Pm* is activated in both XX and XY animals. However, the transcript from Pm requires alternative splicing, dependent on Sxl protein itself, to generate a functional protein. Since only XX animals have early Sxl protein, only XX animals can initiate autoregulatory splicing to produce more functional Sxl protein. In this way Sxl expression can be maintained in a female-specific manner even after dosage compensation has begun.

Several X chromosome transcription factors act as X chromosome counting elements (XCEs) in the soma, and are thought to activate *Sxl Pe* only when two copies are present. In the absence of XCE function, no early Sxl protein is produced and, hence, no functional late Sxl protein can be produced as well. Since Sxl is essential for blocking dosage compensation in females, lack of Sxl leads to an excess of X chromosome gene expression that is lethal. Thus, genes acting as XCEs have a “sisterless (sis)” phenotype, where female progeny fail to survive. The XCEs include the transcription factors SisA, an atypical leucine zipper protein, SisB/Scute, a basic-helix-loop-helix (bHLH) protein, Runt, a Runx family protein,

and SisC/Upd which is a ligand for the JAK/STAT pathway thought to activate *Sxl*. The XCEs act in concert to activate *Sxl Pe* and loss of one copy of any single XCE in females does not cause loss of *Sxl* expression unless combined with other mutations. In addition to the XCEs, there is a maternally supplied activator Daughterless (Da), a bHLH protein that is required for transcriptional activation by SisB/Scute, and negative regulators that are thought to set a threshold for *Sxl* activation, including the HLH protein Deadpan (see [Cline, 1993; Salz and Erickson, 2010] and references therein).

Current evidence indicates that a similar model for Sxl activation occurs in the germline, but with some key differences. Expression of Sxl in the germline is dependent on X chromosome number and is independent of the sexual identity of the surrounding somatic cells (e.g. [Bhaskar et al., 2021]). XX germ cells still express Sxl when present in a male soma, and this is thought to be a major reason why these cells fail in spermatogenesis and are atrophic. The model of autoregulation in the soma, where the early promoter (*Pe*) is active only in females to produce protein that autoregulates the transcript from the maintenance promoter (*Pm*) is also thought to occur in the germline [Hager and Cline, 1997]. cDNA representing the *Pe* transcript was identified in early germ cells [Shigenobu et al., 2006] and expression of an early promoter (*Pe-GFP*) reporter was observed in early first instar larval germ cells [Goyal et al., 2021]. In addition, when the late Sxl protein was specifically tagged, expression was first observed in second instar larvae, after *Pe* is active, consistent with a need to activate *Pe* in order to allow production of functional protein from *Pm* [Goyal et al., 2021].

A key difference between the germline and the soma is in how X chromosomes are counted to activate *Sxl* in the germline. Several genetic experiments indicate that the combination of XCEs is different in the germline than the soma. Germ cells that lack *sisB* can fully function as female germ cells [Steinmann-Zwicky, 1993], as can germ cells that lack Daughterless, the maternal co-factor for *sisB* [Schüpbach, 1985]. Further, compound heterozygotes that simultaneously lack one functional allele of *sisA, sisB, runt* and *Sxl* exhibit a strong defect in the soma, yet such germ cells are fully capable of producing eggs when transplanted into a wild type female [Granadino et al., 1993]. Lastly, the JAK/STAT pathway is activated in the germline in a male-specific manner during early development [Wawersik and Van Doren, 2005], thus, it is unlikely that *sisC/upd* could act as an activator of *Sxl* in the female germline as it does in the soma. These experiments indicate that at least some XCEs used in the soma are not used in the germline. In addition, the *cis*-regulatory regions required for *Pe* activation in the germline are different from those in the soma. A 3.0 kb fragment upstream of *Pe* is sufficient for expression in the soma [Keyes et al., 1992], while a much larger region, including sequences both upstream and downstream from *Pe,* is required for expression in the germline [Goyal et al., 2021]. Despite these differences, it was recently found that one somatic XCE, *sisA,* is utilized in the germline [Goyal et al., 2021]). *sisA* is expressed in early germ cells, and loss of *sisA* in the germline causes a loss of Sxl expression and produces a similar germline defect as loss of *Sxl*. Expression of *Sxl* can rescue *sisA* loss of function, demonstrating that *sisA* is upstream of *Sxl* in the germline [Goyal et al., 2021]. Other activators of *Sxl* in the germline are yet to be discovered.

Targets of Sxl

Another difference between the soma and the germline is that Sxl has different targets in the two cell types. In the soma, there are three known Sxl targets (reviewed by [Salz and Erickson, 2010]. The first is *Sxl* RNA itself, where Sxl binding regulates splicing of the *Sxl Pm* transcript to allow it to produce functional Sxl protein (facilitating the autoregulatory loop described above). In addition, Sxl controls somatic sex determination by regulating RNA splicing of *transformer (tra)* via direct binding of Sxl and other factors. Tra then goes on to regulate which isoform, male or female, of the transcription factor Dsx will be expressed. Lastly, *Sxl* regulates X chromosome dosage compensation by repressing *male-specific lethal-2 (msl-2),* a component of the dosage compensation "MSL complex". At least one other somatic target of Sxl exists, as Sxl has been shown to regulate female egg laying behavior in a Tra-independent manner [Evans and Cline, 2013]. In contrast, while *Sxl* itself is likely a target for Sxl in the germline (for autoregulation), neither *tra* nor *msl-2* are germline targets for *Sxl*. Unlike in the soma, *tra* is not required in the female germline [Marsh and Wieschaus, 1978] and the MSL dosage compensation complex does not function in the germline [Bachiller and Sanchez, 1986; Rastelli and Kuroda, 1998]. While bioinformatic and genomic studies have identified potential germline Sxl targets (e.g. [Robida et al., 2007; Ota et al., 2017; Primus et al., 2019]) to date only a few candidates have been validated and characterized as Sxl targets in the germline.

Tdrd5l.

Recently, transcriptomic analysis was performed on germ cells lacking Sxl to identify novel targets of Sxl in the germline [Primus et al., 2019] and *Tudor-domain containing protein 5-like (Tdrd5l)* was identified as a potential target. Tdrd5l contains a tudor domain and localizes to discrete cytoplasmic punctae that resemble RNA granules that regulate gene expression at the post-transcriptional level. Tdrd5l is highly male-specific in the undifferentiated germline, and is important for male germline sex determination and spermatogenesis [Primus et al., 2019]. The germline defects observed when XX germ cells are present in a male soma (*XX; tra*^{null}) can be partially reversed by over-expressing *Tdrd5l* in the germline [Primus et al., 2019]. *Tdrd5l* appears to be a direct target for regulation by Sxl. Expression of Tdrd5l is dramatically increased in female germ cells upon loss of *Sxl*. Further, mutation of two predicted Sxl binding sites in the *Tdrd5l* transcript lead to a similar upregulation of Tdrd5l expression [Primus et al., 2019]. Interestingly, Tdrd5l is homologous to mouse Tdrd5, which is also required for spermatogenesis [Yabuta et al., 2011].

nanos.

While Sxl is vital to ensuring female identity of XX GSCs, it also plays a role as GSCs begin differentiating and enter oogenesis [Chau et al., 2009]. Sxl exhibits cytoplamsic localization in the GSCs and their early daughters, suggesting a role in regulating translation, while expression is weaker and more nuclear in more differentiated germ cells [Bopp et al., 1993]. *nanos (nos)* is important for female GSC self-renewal and is downregulated during the early stages of germ cell differentiation [Forbes and Lehmann, 1998; Wang and Lin, 2004]. Sxl acts to ensure that Nos is downregulated at the correct place and time, likely in collaboration with the differentiation factor Bag of Marbles (Bam) and

other factors [Chau et al., 2012; Li et al., 2013; Malik et al., 2020]. Nos downregulation fails to occur in the absence of *Sxl* function. Further, Sxl binds to the *nos* mRNA and Nos downregulation is abolished when putative Sxl binding sites in the *nos* mRNA are mutated [Chau et al., 2012]. While this has only subtle effects on proper germline differentiation, *nos* is likely not the only target for Sxl regulation at this stage.

JAK/STAT signaling.—The JAK/STAT pathway is a key determinant of male identity in the germline [Wawersik et al., 2005]. This pathway is activated in male germ cells soon after they first coalesce with somatic cells to form the gonad, and it is essential for male germline gene expression and behavior. At this time, ligands for the JAK/STAT pathway are specifically expressed in the male somatic gonad [Wawersik et al., 2005]. However, the JAK/STAT pathway eventually becomes activated later in the developing ovary, where it is important in somatic cells of the ovary but not in the germline [Decotto and Spradling, 2005]. How this pathway is prevented from activating a male identity in the germline of the ovary remained unknown. However, recent work shows that Sxl-dependent repression of JAK/STAT signaling in female germ cells ensures that JAK/STAT signaling is highly male-specific [Bhaskar et al., 2021]. When XX germ cells are present in a testis, the germline is severely atrophic and germ cells also exhibit greatly decreased JAK/STAT activity relative to XY germ cells. Both the decreased JAK/STAT response and the germline depletion can be partially rescued by removing *Sxl* activity, or by directly activating the JAK/STAT pathway to bypass its repression by Sxl. Conversely, when *Sxl* function is depleted in the germline of the ovary, the germline tumors that form can be rescued by simultaneously reducing JAK/STAT function [Shapiro-Kulnane et al., 2015; Bhaskar et al., 2021]. Together, these data support a model where a normal female germline requires Sxl activity to downregulate JAK/STAT activation [Bhaskar et al., 2021]. However, the specific RNA target(s) by which Sxl represses JAK/STAT signaling remain unknown.

In male germ cells, the JAK/STAT pathway activates *PHD finger protein 7* (*Phf7*). Phf7 is a histone code reader that regulates male germline identity and spermatogenesis [Yang et al., 2012]. Interestingly, the mouse homolog of *Phf7* is also essential for spermatogenesis [Wang et al., 2019]. It is critical that *Phf7* is expressed specifically in males, as expression of *Phf7* in females leads to germline tumors or germline loss [Yang et al., 2012; Shapiro-Kulnane et al., 2015]. Further, *Phf7* is a direct JAK/STAT target gene as loss of STAT, or mutation of STAT binding sites in *Phf7,* lead to loss of *Phf7* expression [Bhaskar et al., 2021]. Phf7 expression is also upregulated in females that lack *Sxl,* and therefore have upregulated JAK/STAT signaling [Shapiro-Kulnane et al., 2015; Bhaskar et al., 2021]. Expression of Phf7 is also controlled by positive autoregulation as well as repression by the H3K9 methylase SETDB1 [Smolko et al., 2018, 2020]. Thus, Phf7 is a key male-determining factor in the germline activated by the JAK/STAT pathway, and it is essential that JAK/STAT, and therefore Phf7, be repressed by Sxl in females.

Dosage compensation in the germline

One open question is whether *Sxl* targets X chromosome dosage compensation in the germline as it does in the soma, in addition to germline sexual identity. Defective dosage compensation could contribute to the germline defects observed when *Sxl* function is

blocked in the female germline. Answering this question is hampered by two important issues. First, as discussed above, the dosage compensation mechanism operating in the soma (the MSL complex) does not act in the germline [Bachiller and Sanchez, 1986; Rastelli and Kuroda, 1998]. Thus, germline dosage compensation would require an independent mechanism from that in the soma that has yet to be discovered, and *Sxl* would have to regulate this mechanism. Second, the question of whether dosage compensation even occurs in the germline has been controversial. Using whole-genome expression profiling, some researchers conclude that dosage compensation occurs in the germline (e.g. [Gupta et al., 2006; Witt et al., 2021]) while others conclude that it does not (e.g. [Meiklejohn et al., 2011; Li et al., 2021; Ota et al., 2021]). Thus, the questions of whether dosage compensation exists in the germline, and whether *Sxl* can regulate this process in an MSL-independent manner, remain to be resolved.

Future Directions

As discussed in this review, *Sxl* is the critical factor specifying germline sexual identity; it is both necessary and sufficient for specifying a female germline fate. However, while progress is being made in both how *Sxl* activity is regulated in a sex-specific manner, and what targets Sxl acts on to regulate germline sexual identity, much remains to be learned. While *sisA* has been identified as a key regulator of *Sxl* in the germline, it is clear that other factors regulating this process remain to be identified. Further, while repression of the JAK/STAT pathway by Sxl is essential for female germline development, how Sxl regulates this pathway remains unknown. In addition, the known targets for Sxl in the germline may not be sufficient to explain all that Sxl does to control germline sexual identity. In particular, we currently know only of ways in which Sxl represses the male pathway in female germ cells, by acting to block *Tdrd5l* and the JAK/STAT pathway. What targets Sxl may activate to promote female germline identity remain undiscovered. Finally, additional targets for Sxl other than *nanos* likely exist as germ cells transition from GSC to enter oogenesis. Thus, a great deal remains to be discovered as to how *Sxl* controls germline sexual identity and development in *Drosophila*. Whether similar RNA binding proteins influence germline sexual development in other species is also of great interest for future work.

Funding Sources:

This work was supported by NIH Grants GM84356 and GM113001 awarded to M.V.D.

References

Bachiller D, and Sanchez L. (1986). Mutations affecting dosage compensation in Drosophila melanogaster: effects in the germ line. Developmental Biology 118, 379–384. [PubMed: 3098595]

Bhaskar PK, Southard S, Baxter K, and Van Doren M. (2021). Germline Sex Determination regulates sex-specific signaling between germline stem cells and their niche. BioRxiv.

Bopp D, Horabin JI, Lersch RA, Cline TW, and Schedl P. (1993). Expression of the Sex-lethal gene is controlled at multiple levels during Drosophila oogenesis. Development 118, 797–812. [PubMed: 8076518]

Camara N, Whitworth C, Dove A, and Van Doren M. (2019). Doublesex controls specification and maintenance of the gonad stem cell niches in Drosophila. Development 146.

Casper A, and Van Doren M. (2006). The control of sexual identity in the Drosophila germline. Development 133, 2783–2791. [PubMed: 16835435]

Chau J, Kulnane LS, and Salz HK (2009). Sex-lethal facilitates the transition from germline stem cell to committed daughter cell in the Drosophila ovary. Genetics 182, 121–132. [PubMed: 19237687]

Chau J, Kulnane LS, and Salz HK (2012). Sex-lethal enables germline stem cell differentiation by down-regulating Nanos protein levels during Drosophila oogenesis. Proc Natl Acad Sci U S A 109, 9465–9470. [PubMed: 22645327]

Cline TW (1993). The Drosophila sex determination signal: how do flies count to two? Trends Genet 9, 385–390. [PubMed: 8310535]

Decotto E, and Spradling AC (2005). The Drosophila ovarian and testis stem cell niches: similar somatic stem cells and signals. Dev Cell 9, 501–510. [PubMed: 16198292]

DeFalco T, Camara N, Le Bras S, and Van Doren M. (2008). Nonautonomous sex determination controls sexually dimorphic development of the Drosophila gonad. Dev Cell 14, 275–286. [PubMed: 18267095]

DeFalco TJ, Verney G, Jenkins AB, McCaffery JM, Russell S, and Van Doren M. (2003). Sex-specific apoptosis regulates sexual dimorphism in the Drosophila embryonic gonad. Dev Cell 5, 205–216. [PubMed: 12919673]

Evans DS, and Cline TW (2013). Drosophila switch gene Sex-lethal can bypass its switch-gene target transformer to regulate aspects of female behavior. Proc Natl Acad Sci U S A 110, E4474–4481. [PubMed: 24191002]

Forbes A, and Lehmann R. (1998). Nanos and Pumilio have critical roles in the development and function of Drosophila germline stem cells. Development 125, 679–690. [PubMed: 9435288]

Fuller MT, and Spradling AC (2007). Male and female Drosophila germline stem cells: two versions of immortality. Science 316, 402–404. [PubMed: 17446390]

Goyal R, Baxter K, and Van Doren M. (2021). sisterless A is required for the activation of Sex lethal in the germline. bioRxiv.

Granadino B, Santamaria P, and Sánchez L. (1993). Sex determination in the germ line of Drosophila melanogaster: activation of the gene Sex-lethal. Development 118, 813–816. [PubMed: 8076519]

Gupta V, Parisi M, Sturgill D, Nuttall R, Doctolero M, Dudko OK, Malley JD, Eastman PS, and Oliver B. (2006). Global analysis of X-chromosome dosage compensation. J Biol 5, 3. [PubMed: 16507155]

Hager JH, and Cline TW (1997). Induction of female Sex-lethal RNA splicing in male germ cells: Implications for Drosophila germline sex determination. Development 124, 5033–5048. [PubMed: 9362474]

Hamada N, Hamazaki N, Shimamoto S, Hikabe O, Nagamatsu G, Takada Y, Kato K, and Hayashi K. (2020). Germ cell-intrinsic effects of sex chromosomes on early oocyte differentiation in mice. PLoS Genet 16, e1008676.

Hashiyama K, Hayashi Y, and Kobayashi S. (2011). Drosophila Sex lethal gene initiates female development in germline progenitors. Science 333, 885–888. [PubMed: 21737698]

Hempel LU, and Oliver B. (2007). Sex-specific DoublesexM expression in subsets of Drosophila somatic gonad cells. BMC Dev Biol 7, 113. [PubMed: 17935627]

Jemc JC (2011). Somatic gonadal cells: the supporting cast for the germline. Genesis 49, 753–775. [PubMed: 21735540]

Keyes LN, Cline TW, and Schedl P. (1992). The primary sex determination signal of Drosophila acts at the level of transcription. Cell 68, 933–943. [PubMed: 1547493]

Li Y, Zhang Q, Carreira-Rosario A, Maines JZ, McKearin DM, and Buszczak M. (2013). Mei-p26 cooperates with Bam, Bgcn and Sxl to promote early germline development in the Drosophila ovary. PLoS One 8, e58301.

Li YR, Lai HW, Huang HH, Chen HC, Fugmann SD, and Yang SY (2021). Trajectory mapping of the early Drosophila germline reveals controls of zygotic activation and sex differentiation. Genome Res 31, 1011–1023. [PubMed: 33858841]

Malik S, Jang W, Kim JY, and Kim C. (2020). Mechanisms ensuring robust repression of the Drosophila female germline stem cell maintenance factor Nanos via posttranscriptional regulation. FASEB J 34, 11421–11430. [PubMed: 32654316]

Marsh JL, and Wieschaus E. (1978). Is sex determination in germ line and soma controlled by separate genetic mechanisms? Nature 272, 249–251. [PubMed: 628449]

Meiklejohn CD, Landeen EL, Cook JM, Kingan SB, and Presgraves DC (2011). Sex chromosome-specific regulation in the Drosophila male germline but little evidence for chromosomal dosage compensation or meiotic inactivation. PLoS Biol 9, e1001126.

Ota R, Hayashi M, Morita S, Miura H, and Kobayashi S. (2021). Absence of X-chromosome dosage compensation in the primordial germ cells of Drosophila embryos. Sci Rep 11, 4890. [PubMed: 33649478]

Ota R, Morita S, Sato M, Shigenobu S, Hayashi M, and Kobayashi S. (2017). Transcripts immunoprecipitated with Sxl protein in primordial germ cells of Drosophila embryos. Dev Growth Differ 59, 713–723. [PubMed: 29124738]

Poirie M, Niederer E, and Steinmann-Zwicky M. (1995). A sex-specific number of germ cells in embryonic gonads of Drosophila. Development 121, 1867–1873. [PubMed: 7601000]

Primus S, Pozmanter C, Baxter K, and Van Doren M. (2019). Tudor-domain containing protein 5-like promotes male sexual identity in the Drosophila germline and is repressed in females by Sex lethal. PLoS Genet 15, e1007617.

Rastelli L, and Kuroda MI (1998). An analysis of maleless and histone H4 acetylation in Drosophila melanogaster spermatogenesis. Mech Dev 71, 107–117. [PubMed: 9507080]

Robida MD, Rahn A, and Singh R. (2007). Genome-wide identification of alternatively spliced mRNA targets of specific RNA-binding proteins. PLoS One 2, e520. [PubMed: 17565373]

Salz HK, and Erickson JW (2010). Sex determination in Drosophila: The view from the top. Fly (Austin) 4, 60–70. [PubMed: 20160499]

Schüpbach T. (1985). Normal female germ cell differentiation requires the female X chromosome to autosome ratio and expression of sex-lethal in Drosophlia melanogaster. Genetics 109, 529–548. [PubMed: 3920120]

Shapiro-Kulnane L, Smolko AE, and Salz HK (2015). Maintenance of Drosophila germline stem cell sexual identity in oogenesis and tumorigenesis. Development 142, 1073–1082. [PubMed: 25758221]

Sheng XR, Posenau T, Gumulak-Smith JJ, Matunis E, Van Doren M, and Wawersik M. (2009). Jak-STAT regulation of male germline stem cell establishment during Drosophila embryogenesis. Dev Biol 334, 335–344. [PubMed: 19643104]

Shigenobu S, Arita K, Kitadate Y, Noda C, and Kobayashi S. (2006). Isolation of germline cells from Drosophila embryos by flow cytometry. Dev Growth Differ 48, 49–57. [PubMed: 16466393]

Smolko AE, Shapiro-Kulnane L, and Salz HK (2018). The H3K9 methyltransferase SETDB1 maintains female identity in Drosophila germ cells. Nat Commun 9, 4155. [PubMed: 30297796]

Smolko AE, Shapiro-Kulnane L, and Salz HK (2020). An autoregulatory switch in sex-specific phf7 transcription causes loss of sexual identity and tumors in the Drosophila female germline. Development 147.

Staab S, Heller A, and Steinmann-Zwicky M. (1996). Somatic sex-determining signals act on XX germ cells in Drosophila embryos. Development 122, 4065–4071. [PubMed: 9012526]

Steinmann-Zwicky M. (1993). Sex determination in Drosophila: sis-b, a major numerator element of the X:A ratio in the soma, does not contribute to the X:A ratio in the germ line. Development 117, 763–767. [PubMed: 8330539]

Steinmann-Zwicky M, Schmid H, and Nöthiger R. (1989). Cell-autonomous and inductive signals can determine the sex of the germ line of Drosophila by regulating the gene Sxl. Cell 57, 157–166. [PubMed: 2702687]

Van Deusen EB (1977). Sex determination in germ line chimeras of Drosophila melanogaster. J Embryol Exp Morphol 37, 173–185. [PubMed: 404385]

Wang X, Kang JY, Wei L, Yang X, Sun H, Yang S, Lu L, Yan M, Bai M, Chen Y, et al. (2019). PHF7 is a novel histone H2A E3 ligase prior to histone-to-protamine exchange during spermiogenesis. Development 146.

Wang Z, and Lin H. (2004). Nanos maintains germline stem cell self-renewal by preventing differentiation. Science 303, 2016–2019. [PubMed: 14976263]

Wawersik M, Milutinovich A, Casper AL, Matunis E, Williams B, and Van Doren M. (2005). Somatic control of germline sexual development is mediated by the JAK/STAT pathway. Nature 436, 563–567. [PubMed: 16049490]

Wawersik M, and Van Doren M. (2005). nanos is required for formation of the spectrosome, a germ cell-specific organelle. Dev Dyn.

Witt E, Shao Z, Hu C, Krause HM, and Zhao L. (2021). Single-cell RNA-sequencing reveals pre-meiotic X-chromosome dosage compensation in Drosophila testis. PLoS Genet 17, e1009728.

Yabuta Y, Ohta H, Abe T, Kurimoto K, Chuma S, and Saitou M. (2011). TDRD5 is required for retrotransposon silencing, chromatoid body assembly, and spermiogenesis in mice. J Cell Biol 192, 781–795. [PubMed: 21383078]

Yang SY, Baxter EM, and Van Doren M. (2012). Phf7 controls male sex determination in the Drosophila germline. Dev Cell 22, 1041–1051. [PubMed: 22595675]

Yuan H, and Yamashita YM (2010). Germline stem cells: stems of the next generation. Curr Opin Cell Biol 22, 730–736. [PubMed: 20817500]

Zarkower D, and Murphy MW (2021). DMRT1: An Ancient Sexual Regulator Required for Human Gonadogenesis. Sex Dev, 1–14. [PubMed: 34649256]