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Sex Determination in Insects: a binary decision based on alternative splicing

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Abstract

The gene regulatory networks that control sex determination vary between species. Despite these differences, comparative studies in insects have found that alternative splicing is reiteratively used in evolution to control expression of the key sex determining genes. Sex determination is best understood in *Drosophila* where activation of the RNA binding protein encoding gene *Sex-lethal* is the central female-determining event. *Sex-lethal* serves as a genetic switch because once activated it controls its own expression by a positive feedback splicing mechanism. Sex fate choice in is also maintained by self-sustaining positive feedback splicing mechanisms in other dipteran and hymenopteran insects, although different RNA binding protein encoding genes function as the binary switch. Studies exploring the mechanisms of sex-specific splicing have revealed the extent to which sex determination is integrated with other developmental regulatory networks.

Introduction

The gene regulatory networks that control sex determination vary tremendously between species and are among the most rapidly evolving networks in all of biology. In insects alone, marked variation exists in the primary chromosomal signals that determine sex (reviewed in [1–3]). For example, in some Diptera, such as *Drosophila melanogaster*, a chromosome counting mechanism is used: two X chromosomes signals female development and one X chromosome yields male development. In other Diptera, such as the distantly related housefly *Musca domestica* and the medfly *Ceratitis capitata*, the presence or absence of the Y chromosome determines sex. In Hymenoptera, such as the honeybee *Apis mellifera* and the wasp *Nasonia vitripennis*, sex is determined by a haplodiploid mechanism in which males emerge from unfertilized eggs and females from fertilized eggs. Despite this wide variety of initiating mechanisms, positive feedback alternative splicing loops have emerged as the key molecular strategy for maintaining sexual fate.

In this review I discuss the reiterative use of alternative splicing cascades to determine sex in insects. Since the *Drosophila* sex determination system is the gold standard to which other insects are compared, I will first review recent advances in our understanding of how *Sex-lethal* (*Sxl*) serves as the heritable switch gene by controlling its own splicing. I will then

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provide examples of how the RNA binding protein SXL renders the cell capable of initiating the cascade of post-transcriptional events that ultimately lead to sexual dimorphism. Lastly I will discuss the unexpected molecular parallels between the sex-determining positive feedback splicing mechanisms used by other insects where *transformer (tra)* occupies *Sxl*'s position as the binary switch gene.

The sex fate choice decision in *Drosophila*: the *Sxl* autoregulatory splicing loop

In *Drosophila melanogaster*, the choice to be male or female is made early in embryogenesis when the X chromosome number is relayed through regulatory proteins such that *Sxl* is activated only in XX animals (reviewed in [4]). Despite its importance, the X counting mechanism functions only transiently and female identity is maintained by a self-sustaining positive feedback mechanism in which *Sxl* controls its own splicing. In the absence of SXL protein, as in XY cells, all *Sxl* transcripts include the translation-terminating third exon and a noncoding mRNA is produced. If SXL protein is present, as in XX cells, the third male-specific exon is skipped, thereby generating only protein-encoding mRNAs. Stable SXL expression is the pivotal female-determining event as it controls, either directly or indirectly, all aspects of sexual dimorphism.

Genetic studies have established that SXL protein is both necessary and sufficient to engage the *Sxl* autoregulatory splicing loop [5, 6]. The SXL protein contains two highly conserved RRM-type RNA binding domains. Accordingly, all of SXL's biological functions are believed to be the result of its ability to recognize and selectively bind to sequences with long poly(U) stretches interrupted by guanines. The *Sxl* pre-mRNA contains several sets of putative SXL binding sets, the most critical of which are located in the introns >200 nucleotides downstream and >200 nucleotides upstream of the regulated male exon [7]. Although recognition of the appropriate binding site is essential, efficient exon skipping requires that SXL interacts with several general splicing factors, including the U1 snRNP (Table 1). Interestingly, when ChIP analysis was used to visualize the co-transcriptional recruitment of SXL and the U1 snRNP along the *Sxl* gene in female embryos, it was found that SXL does not interfere with the deposition of the U1 snRNP at the male exon 5' splice site [8•]. These data, together with earlier genetic and biochemical studies [9–11], support a model in which the spliceosome begins to assemble on the male exon in both males and females. In females, however, SXL prevents the reaction from finishing by interacting with and repressing the function of these general splicing factors. The reaction could be blocked immediately or spliceosome assembly could continue, stalling only later in the pathway. The end result is a dead-end complex that guarantees that the male exon is skipped, and that exon 2 is spliced to exon 4 (Figure 1).

Although components of the spliceosome clearly play a direct role in SXL-mediated splicing inhibition, a recent study hints that communication between chromatin and RNA is also important [8•]. In this study, the *Drosophila* protein most closely related to the yeast histone H3K4me3 binding protein BYE1, called Protein Partner of Snf (PPS), was identified as part of the machinery required for *Sxl* male exon skipping. Consistent with its role in splicing, PPS interacts with the U1 snRNP, the SXL protein, and the *Sxl* pre-mRNA. Furthermore, ChIP studies indicate that PPS is likely to be loaded onto the *Sxl* RNA by the transcription machinery. Whether PPS is in fact associated with the transcription machinery and/or is a chromatin binding protein remains to be determined. However, in light of the well documented influence of chromatin structure and transcription on the pattern of alternative splicing (reviewed in [12]), it is tempting to speculate that PPS functions as an adaptor protein to ensure that the male exon is skipped, perhaps by accelerating the

recruitment of SXL to the nascent transcript or by facilitating the formation of the inhibitory SXL/U1 snRNP interaction.

Although speculative, a mechanism integrating chromatin structure, transcription and splicing is likely to be especially important when the *Sxl* autoregulatory loop is first established in the early embryo (Figure 2). Engagement of the autoregulatory loop requires an initiating source of SXL protein and the coordinated regulation of two *Sxl* promoters: *SxlPe* (the establishment promoter) and *SxlPm* (the maintenance promoter). The first promoter to be activated, *SxlPe*, is transiently expressed during the syncytial blastoderm stage in response to two X-chromosomes. The SXL protein produced from the *SxlPe* derived transcripts is then present when *SxlPm* is first activated and serves to drive the initiating round of exon skipping. It is now clear that the changeover from *SxlPe* to *SxlPm* is precisely timed, and that disruptions in the chronology of these events interfere with the correct transmission of X-chromosome number to *Sxl* [13, 14•]. It would not be surprising if the machinery that controls the timing of *SxlPm* activation also participates in the decision to include or skip the male exon.

Once activated, *Sxl* is capable of initiating several different regulatory cascades leading to sexual dimorphism and dosage compensation. Although these regulatory cascades are traditionally viewed as unidirectional, recent genetic studies suggest that *Sxl* expression is subject to positive reinforcement from one of its immediate downstream target genes, *transformer (tra)* [15•]. Whether this effect is direct has not been tested, however the fact that TRA is an RNA binding protein and a tandem pair of TRA binding sites are found in the intron upstream of the male exon is suggestive of a direct effect. This retrograde splicing mechanism might stabilize and/or augment the decision to skip the third exon in females, thereby locking in the female-determining program.

Execution of the sex fate choice in *Drosophila*: *Sxl*-mediated gene regulation

The execution of all female-specific regulatory programs depend, directly or indirectly, on SXL protein expression (reviewed in [4, 16]). Two sets of recent studies emphasize the extent to which sex-specific splicing regulatory networks are intertwined with tissue-specific regulatory networks.

The gene regulatory network that leads to most, but not all, sexually dimorphic characteristics and behavior begins with SXL-directed *tra* splicing. Expression of the female specific RNA binding protein TRA in turn controls splicing of *doublesex (dsx)* and *fruitless (fru)* to produce sex-specific transcription factors that ultimately control most aspects of sexual differentiation and behavior. However, to say that the activity of *dsx* and *fru* is entirely controlled by the *Sxl*→*tra* pathway is a gross oversimplification, as *dsx* and *fru* are subject to an additional layer of highly restrictive tissue-specific transcriptional control [17–20•]. Thus, even though ubiquitous SXL protein expression provides all cells with the “knowledge” that they are female, execution of the *dsx/fru* program is restricted to specific cell types by the availability of the pre-mRNA substrates for TRA-mediated splicing regulation.

A second example where tissue-specific and sex-specific regulation are integrated comes from studies showing that *Sxl* regulates Notch signaling to generate sexually dimorphic characteristics such as the number of neurosensory bristles on the A5 abdominal sternite [21]. In this case, SXL does not act through *tra*, but directly controls *Notch* by regulating its translation. Notch is used reiteratively during development to specify various cell fates, but most specification events are not sexually dimorphic. Therefore, it is likely that sex-specific

Notch regulation is restricted to specific cell types, whereas the majority of Notch regulation is monomorphic. The mechanism that restricts sex-specific modulation of Notch signaling remains to be elucidated. Genetic studies carried out in the wing disc, however, suggest that Hrp48, a member of the hnRNP family of RNA binding proteins, is required to protect *Notch* mRNAs from SXL-mediated translational repression [22, 23]. Biochemical studies should clarify whether Hrp48 acts on the *Notch* mRNA substrate to restrict its availability for SXL-mediated regulation or whether Hrp48 influences SXL activity directly.

Shared regulatory logic amid evolutionary diversity

Starting with *Drosophila melanogaster* as the source of comparative information, molecular phylogenetic studies have revealed both evolutionary conservation and divergence among the regulatory pathways that control sex in insects. For example, divergence at the top of the pathway is evident, as *Sxl*'s sex-specific expression pattern and function extends only to its closest sibling species [24, 25]. The sex-specific splicing regulation of *dsx* and *fru*, on the other hand, is highly conserved in a broad range of insects (reviewed in [26, 27]). Strikingly, there is a high degree of conservation in sequence elements located in and around the regulated splice sites that include putative TRA binding sites. This suggests that the evolutionary conservation extends to the molecular mechanism that controls *dsx* and *fru* splice site usage. Together with RNAi knockdown experiments that demonstrate functional conservation across a diverse sampling of insects, these phylogenetic studies suggest an ancient origin and role for *dsx* and *fru* in sex determination.

Despite the fact that *dsx* and *fru* homologs contain conserved sets of TRA binding sites, the TRA protein itself is poorly conserved. Indeed, TRA has been labeled as one of the more rapidly evolving members of the SR family of RNA binding proteins (see for example Figure S2 in [28•]). The lack of sequence conservation while retaining RNA binding specificity can be explained by the co-evolution between TRA and its putative binding partner TRA-2. In *Drosophila* TRA function requires an interaction with the non-sex specific TRA-2 RNA binding protein. While it is not known whether TRA-2 is an obligate binding partner in other insects, a conserved role for TRA-2 in sex specific splicing is supported by the observation that *tra-2* homologs are required for *dsx* and *fru* splicing [29–31].

A role for *tra* as the sex-determination switch gene was first suggested by studies in the medfly *Ceratitis capitata* where *Cctra* was found to be required for its own splicing [30, 32, 33]. Interestingly, the *tra* genomic structure differs considerably between *Drosophila* and *Ceratitis*. In fact, the *Cctra* architecture is more similar to that of *Sxl*, with translation terminating male-specific exons that are sex-specifically skipped to produce protein encoding mRNAs (Figure 3). *tra* homologs have also been identified as the sex switch gene in a number of other insects, including the distantly related housefly *Musca domestica*, honeybee *Apis mellifera* and wasp *Nasonia vitripennis* [28•, 34•, 35, 36]. In each case translation-terminating male exons form the backbone of an autoregulatory splicing mechanism for maintaining sexual fate (Figure 3). The recurrence of exon skipping in this context leads us to speculate that alternative splicing control is an evolutionarily advantageous strategy for positive feedback regulatory mechanisms.

The central unanswered question with regard to cell fate determination is how the decision whether or not to activate the positive feedback splicing loop is made. In both the medfly and the housefly, it is the presence or absence of the Y chromosome that determines sex, not X-chromosome number. This implies that there is both a Y-encoded product that prevents the initiation of the *tra* autoregulatory loop in males and a mechanism that provides the initiating source of TRA protein in females. The presence of maternally provided *tra* mRNA

in both male and female embryos, suggests that an initiating source of TRA protein is maternally provided to all embryos and that male development depends on a Y-encoded mechanism that interferes with establishment of the autoregulatory loop [28•, 37].

In Hymenoptera, such as the honeybee *Apis mellifera* and the wasp *Nasonia vitripennis*, sex is determined by a haplodiploid mechanism in which males emerge from unfertilized eggs and females from fertilized eggs. Recent studies in the wasp have shown that while maternal *tra* mRNA is likely to provide an initiating source of TRA protein to both haploid and diploid embryos, zygotic *tra* pre-mRNA transcription is limited to diploid embryos [34•]. Haploid embryos are therefore deprived of pre-mRNA substrate during this critical period of development and the splicing loop is not engaged. Although the mechanism that restricts *tra* transcription to diploids remains to be discovered, genomic imprinting is a potential explanation as it mediates many parent of origin effects.

Concluding Remarks

Together these comparative studies illustrate that positive feedback splicing mechanisms are used reiteratively in evolution to maintain sexual fate. Future studies focused on the similarities and differences in how self-regulation is accomplished and how downstream target gene expression is modulated will expand our understanding of the organization, function and evolution of the RNA splicing network that controls sex determination. It will be particularly interesting to determine how much variability is tolerated while equivalent homologous functions are still maintained in evolution. In addition, much remains to be learned about how the self-regulating binary switch genes are activated in early embryogenesis. In all insects examined thus far, including *Drosophila*, activation of the autoregulatory splicing loop is tightly correlated with the events leading to formation of the cellular blastoderm and the maternal to zygotic transition. Achieving a deeper understanding of how sex-specific splicing regulation is connected to the more general regulatory events occurring during this dynamic period of development will be a challenge for the field in the coming years.

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References and recommended reading

- of special interest
 - of outstanding interest
1. Verhulst EC, van de Zande L, Beukeboom LW. Insect sex determination: it all evolves around transformer. *Curr Opin Genet Dev.* 2010; 20:376–383. [PubMed: 20570131]
 2. Gempe T, Beye M. Function and evolution of sex determination mechanisms, genes and pathways in insects. *Bioessays.* 2011; 33:52–60. [PubMed: 21110346]
 3. Bopp D. About females and males: continuity and discontinuity in flies. *J Genet.* 2010; 89:315–323. [PubMed: 20876998]
 4. Salz HK, Erickson JW. Sex determination in *Drosophila*: The view from the top. *Fly (Austin).* 2010; 4:60–70. [PubMed: 20160499]
 5. Bell LR, Horabin JI, Schedl P, Cline TW. Positive autoregulation of *Sex-lethal* by alternative splicing maintains the female determined state in *Drosophila*. *Cell.* 1991; 65:229–239. [PubMed: 2015624]

6. Cline TW. Autoregulatory functioning of a *Drosophila* gene product that establishes and maintains the sexually determined state. *Genetics*. 1984; 107:231–277. [PubMed: 6735170]
7. Horabin JI, Schedl P. *Sex-lethal* autoregulation requires multiple cis-acting elements upstream and downstream of the male exon and appears to depend largely on controlling the use of the male exon 5' splice site. *Mol Cell Biol*. 1993; 13:7734–7746. [PubMed: 8246990]
- 8•. Johnson ML, Nagengast AA, Salz HK. PPS, a large multidomain protein, functions with *Sex-lethal* to regulate alternative splicing in *Drosophila*. *PLoS Genet*. 2010; 6:e1000872. [PubMed: 20221253] This paper identifies PPS, a likely chromatin binding protein, as a key player in *Sxl* autoregulatory splicing. The data suggest a functional link between transcription and SXL-mediated alternative splicing.
9. Nagengast AA, Stitzinger SM, Tseng C-H, Mount SM, Salz HK. *Sex-lethal* splicing autoregulation in vivo: interactions between SEX-LETHAL, the U1 snRNP and U2AF underlie male exon skipping. *Development*. 2003; 130:463–471. [PubMed: 12490553]
10. Chaouki AS, Salz HK. *Drosophila* SPF45: a bi-functional protein with roles in both splicing and DNA repair. *PLoS Genet*. 2006; 2:e178. [PubMed: 17154718]
11. Penn JK, Graham P, Deshpande G, Calhoun G, Chaouki AS, Salz HK, Schedl P. Functioning of the *Drosophila* Wilms'-tumor-1-associated protein homolog, Fl(2)d, in Sex-lethal-dependent alternative splicing. *Genetics*. 2008; 178:737–748. [PubMed: 18245840]
12. Luco RF, Allo M, Schor IE, Kornblihtt AR, Misteli T. Epigenetics in Alternative Pre-mRNA Splicing. *Cell*. 2011; 144:16–26. [PubMed: 21215366]
13. Erickson JW, Quintero JJ. Indirect effects of ploidy suggest X chromosome dose, not the X:A ratio, signals sex in *Drosophila*. *PLoS Biol*. 2007; 5:e332. [PubMed: 18162044]
- 14•. Gonzalez AN, Lu H, Erickson JW. A shared enhancer controls a temporal switch between promoters during *Drosophila* primary sex determination. *Proc Natl Acad Sci U S A*. 2008; 105:18436–18441. [PubMed: 19011108] First demonstration that the *SxlPm* promoter is activated earlier in females than in males and that its expression overlaps briefly with that of *SxlPe* during the syncytial blastoderm stage.
- 15•. Siera SG, Cline TW. Sexual back talk with evolutionary implications: stimulation of the *Drosophila* sex-determination gene *Sex-lethal* by its target *transformer*. *Genetics*. 2008; 180:1963–1981. [PubMed: 18845845] This paper shows that *transformer* may contribute to *Sxl* alternative splicing control.
16. Camara N, Whitworth C, Van Doren M. The creation of sexual dimorphism in the *Drosophila* soma. *Curr Top Dev Biol*. 2008; 83:65–107. [PubMed: 19118664]
17. Rideout EJ, Billeter JC, Goodwin SF. The sex-determination genes *fruitless* and *doublesex* specify a neural substrate required for courtship song. *Curr Biol*. 2007; 17:1473–1478. [PubMed: 17716899]
- 18•. Hempel LU, Oliver B. Sex-specific DoublesexM expression in subsets of *Drosophila* somatic gonad cells. *BMC Dev Biol*. 2007; 7:113. [PubMed: 17935627] See reference [20•].
- 19•. Rideout EJ, Dornan AJ, Neville MC, Eadie S, Goodwin SF. Control of sexual differentiation and behavior by the *doublesex* gene in *Drosophila melanogaster*. *Nat Neurosci*. 2010; 13:458–466. [PubMed: 20305646] See reference [20•].
- 20•. Robinett CC, Vaughan AG, Knapp JM, Baker BS. Sex and the single cell. II. There is a time and place for sex. *PLoS Biol*. 2010; 8:e1000365. [PubMed: 20454565] Together with references [18•, 19•], this study provides an elegant demonstration that sex-specific splicing regulation is integrated with tissue-specific regulation.
21. Penn JK, Schedl P. The master switch gene *Sex-lethal* promotes female development by negatively regulating the N-signaling pathway. *Dev Cell*. 2007; 12:275–286. [PubMed: 17276344]
22. Suissa Y, Ordan E, Deshpande G, Gerlitz O. Males and females: Creating differences while maintaining the similarities. *Fly (Austin)*. 2011; 5:25–28. [PubMed: 21057219]
23. Suissa Y, Kalifa Y, Dinur T, Graham P, Deshpande G, Schedl P, Gerlitz O. *Hrp48* attenuates *Sxl* expression to allow for proper *Notch* expression and signaling in wing development. *Proc Natl Acad Sci U S A*. 2010; 107:6930–6935. [PubMed: 20351283]
24. Traut W, Niimi T, Ikeo K, Sahara K. Phylogeny of the sex-determining gene *Sex-lethal* in insects. *Génome*. 2006; 49:254–262.

25. Cline TW, Dorsett M, Sun S, Harrison MM, Dines J, Sefton L, Megna L. Evolution of the *Drosophila* feminizing switch gene *Sex-lethal*. *Genetics*. 2010; 186:1321–1336. [PubMed: 20837995]
26. Shukla JN, Nagaraju J. *Doublesex*: a conserved downstream gene controlled by diverse upstream regulators. *J Genet*. 2010; 89:341–356. [PubMed: 20877001]
27. Salvemini M, Polito C, Saccone G. *fruitless* alternative splicing and sex behaviour in insects: an ancient and unforgettable love story? *J Genet*. 2010; 89:287–299. [PubMed: 20876995]
- 28••. Hediger M, Henggeler C, Meier N, Perez R, Saccone G, Bopp D. Molecular characterization of the key switch F provides a basis for understanding the rapid divergence of the sex-determining pathway in the housefly. *Genetics*. 2010; 184:155–170. [PubMed: 19841093] This paper provides the first demonstration that *tra* is the binary switch in the housefly. Furthermore, the absence of TRA protein in the unfertilized eggs from *Arrhenogenic* mutant mothers suggests that the initiating source of TRA protein is maternally provided.
29. Burghardt G, Hediger M, Siegenthaler C, Moser M, Dubendorfer A, Bopp D. The *transformer2* gene in *Musca domestica* is required for selecting and maintaining the female pathway of development. *Dev Genes Evol*. 2005; 215:165–176. [PubMed: 15662529]
30. Salvemini M, Robertson M, Aronson B, Atkinson P, Polito L, Saccone G. *Ceratitis capitata transformer-2* gene is required to establish and maintain the autoregulation of *Cctra*, the master gene for female sex determination. *Int J Dev Biol*. 2009; 53:109–120. [PubMed: 19123132]
31. Sarno F, Ruiz MF, Eirín-López JM, Perondini AL, Selivon D, Sánchez L. The gene *transformer-2* of *Anastrepha* fruit flies (Diptera, Tephritidae) and its evolution in insects. *BMC Evol Biol*. 2010; 10:140. [PubMed: 20465812]
32. Pane A, Salvemini M, Delli Bovi P, Polito C, Saccone G. The *transformer* gene in *Ceratitis capitata* provides a genetic basis for selecting and remembering the sexual fate. *Development*. 2002; 129:3715–3725. [PubMed: 12117820]
33. Pane A, De Simone A, Saccone G, Polito C. Evolutionary conservation of *Ceratitis capitata transformer* gene function. *Genetics*. 2005; 171:615–624. [PubMed: 15998727]
- 34••. Verhulst EC, Beukeboom LW, van de Zande L. Maternal control of haplodiploid sex determination in the wasp *Nasonia*. *Science*. 2010; 328:620–623. [PubMed: 20431014] First study to suggest the diploid-specific *tra* activation is regulated by an imprinting mechanism in which transcription of the *tra* pre-mRNA substrate is restricted to diploid embryos.
35. Hasselmann M, Gempe T, Schiott M, Nunes-Silva CG, Otte M, Beye M. Evidence for the evolutionary nascence of a novel sex determination pathway in honeybees. *Nature*. 2008; 454:519–522. [PubMed: 18594516]
36. Gempe T, Hasselmann M, Schiøtt M, Hause G, Otte M, Beye M. Sex Determination in Honeybees: Two Separate Mechanisms Induce and Maintain the Female Pathway. *PLoS Biol*. 2009; 7:e1000222. [PubMed: 19841734]
37. Gabrieli P, Falaguerra A, Siciliano P, Gomulski LM, Scolari F, Zacharopoulou A, Franz G, Malacrida AR, Gasperi G. Sex and the single embryo: early development in the Mediterranean fruit fly, *Ceratitis capitata*. *BMC Dev Biol*. 2010; 10:12. [PubMed: 20102629]
38. Horabin JJ, Schedl P. Splicing of the *Drosophila Sex-lethal* early transcripts involves exon skipping that is independent of Sex-lethal protein. *Rna*. 1996; 2:1–10. [PubMed: 8846292]
39. Zhu C, Urano J, Bell LR. The *Sex-lethal* early splicing pattern uses a default mechanism dependent on the alternative 5' splice sites. *Mol Cell Biol*. 1997; 17:1674–1681. [PubMed: 9032294]
40. Salz HK, Mancebo RSY, Nagengast AA, Speck O, Psotka M, Mount SM. The *Drosophila* U1-70K protein is required for viability, but its arginine-rich domain is dispensable. *Genetics*. 2004; 168:2059–2065. [PubMed: 15611175]
41. Flickinger TW, Salz HK. The *Drosophila* sex determination gene *snf* encodes a nuclear protein with sequence and functional similarity to the mammalian U1A snRNP protein. *Genes Dev*. 1994; 8:914–925. [PubMed: 7926776]
42. Lallena MJ, Chalmers KJ, Llamazares S, Lamond AI, Valcarcel J. Splicing Regulation at the Second Catalytic Step by Sex-lethal Involves 3' Splice Site Recognition by SPF45. *Cell*. 2002; 109:285–296. [PubMed: 12015979]

43. Ortega A, Niksic M, Bachi A, Wilm M, Sanchez L, Hastie N, Valcarcel J. Biochemical function of female-lethal (2)D/Wilms' tumor suppressor-1-associated proteins in alternative pre-mRNA splicing. *J Biol Chem.* 2003; 278:3040–3047. [PubMed: 12444081]

Figure 1. *Sxl* splicing regulation in *Drosophila*

Schematic illustrating the portion of the *Sxl* gene that gives rise to sex-specific transcripts throughout most of development and into adulthood. In both males and females, the spliceosome begins to assemble on the male specific exon (exon 3, in red) with the binding of the U1 snRNP to the 5' splice site and the binding of the U2AF/SPF45 proteins near the 3' splice site. In females, SXL binds to sequences in the flanking introns and interacts with several general splicing factors, including the U1 snRNP, the U2AF complex, SPF45, FL2(d) (not shown) and Vir (not shown). The end result is a dead-end complex that guarantees that the male exon will be skipped and that exon 2 is spliced to exon 4. In males, where there is no SXL protein, the spliceosome continues to assemble and the translation-terminating male exon is included in the mature transcript.

Figure 2. Regulatory logic that underlies stable SXL protein expression in XX *Drosophila* embryos

(Top) Schematic illustrating the portion of the *Sxl* gene that gives rise to sex-specific transcripts through the differential use of two promoters and alternative splicing. The early embryo-specific exon (E1) is in blue. The mechanism that drives splicing of the *SxlPe* pre-mRNAs such that exon E1 is joined directly to exon 4 is not understood [38, 39]. The first exon used throughout most of development is in black (L1) and the translation terminating male-specific third exon is in red. Other exons are in gray. (Bottom) Overview of the regulatory steps that lead to stable establishment of the autoregulatory splicing loop. During the syncytial blastoderm stage, *SxlPe* transcription is activated in animals with two X chromosomes. The SXL protein produced from the *SxlPe* transcripts directs the splicing of the newly transcribed RNA from the *SxlPm* promoter. *SxlPe* is shut down during cellularization, and SXL protein continues to be produced from the *SxlPm* transcripts.

Figure 3. Phylogenetic comparison of *tra* gene structure and regulation

(Left) The evolutionary relationship of the species discussed in this review. (Right) Schematic illustrating the portion of the *tra* gene that is alternatively spliced to give rise to sex-specific products. The male-specific translation terminating male exons is in red. For each transcript, the lines above correspond to the female-specific splicing pattern and the lines below correspond to the male-specific splicing pattern. Note that the *tra* orthologs exhibit different types of alternative splicing regulation, including alternative 3' splice site selection, cassette exon skipping and 5' splice site selection.

Table 1General splicing factors required for *Sxl* male exon skipping

Gene *	Human ortholog *	Biochemical role(s)	References
<i>U1-70K</i>	U1-70K	U1 snRNP component & 5' splice site recognition	[9, 40]
<i>sans-fille (snf)</i>	U1A/U2B"	U1 snRNP & U2 snRNP component	[9, 41]
<i>SPF45</i>	SPF45 (RBM17)	3' splice site recognition & U2 snRNP recruitment	[10, 42]
<i>U2AF-50</i>	U2AF-65	3' splice site recognition & U2 snRNP recruitment	[9, 42]
<i>U2AF-38</i>	U2AF-35	3' splice site recognition & U2 snRNP recruitment	[9, 42]
<i>fl(2)d</i>	WTAP	unknown/present in purified spliceosome	[11, 43]

* Gene name abbreviations and protein name synonyms are listed in parentheses.