Sex determination: **Co-opted signals determine gender**

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The *Drosophila* JAK–STAT pathway and its ligand Unpaired are required for a wide range of developmental processes. Recent results have identified Unpaired as an activator of *sex-lethal* and revealed a new role for the JAK–STAT pathway in sex determination.

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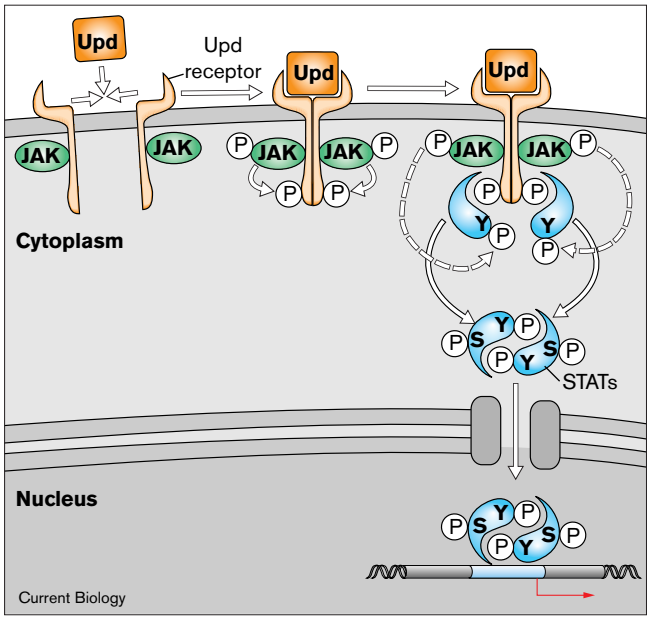
Evolution has created a diverse spectrum of mechanisms for determining sexual identity in different organisms, and the molecular basis of these mechanisms is now beginning to be understood in some systems. In the fruitfly *Drosophila melanogaster*, one of the molecules central to the process of sex determination is the Sex-lethal protein.

Figure 1



The *Drosophila* JAK–STAT pathway consists of the extracellular ligand Unpaired (Upd), a transmembrane receptor and an associated JAK kinase, as well as the normally cytosolic STAT molecules. Following ligand binding and receptor activation, both receptor and JAK molecules are phosphorylated (-P), leading to recruitment of STAT molecules which are themselves activated by phosphorylation. Dimerised STAT molecules then translocate to the nucleus where they bind a consensus DNA target site and activate transcription.

Using a system of transcriptional activators encoded by genes on the X chromosome, and a repressor encoded by an autosomal gene, initial expression of the *sex-lethal* gene is turned on in future females (XX), while it remains off in males (XY). Two recent studies [1,2] have found that one of the X-chromosomal activating genes, called *sisterless-C*, is identical to *unpaired*, which encodes a secreted ligand known to activate the JAK–STAT pathway [3]. Both Unpaired and the downstream components JAK — for 'Janus kinase' — and STAT — for 'signal transducer and activator of transcription' — are required for *sex-lethal* expression.

The JAK–STAT pathway was initially identified by its role in interleukin and cytokine signalling in vertebrate systems, and studies of gene knockout mice have shown that the pathway plays a crucial role in haematopoiesis [4]. The conserved JAK–STAT pathway in *Drosophila* is involved in several developmental processes: not only is it required during haematopoiesis, but JAK–STAT signalling is necessary for the correct expression of a number of members of the 'pair-rule' class of segmentation genes, including *even-skipped* and *runt*, part of the complex hierarchy of interacting genes that divide the developing embryo into segments [5,6]. In addition to these early requirements, the JAK–STAT pathway also functions in the adult eye during ommatidial rotation [5].

The known components of the *Drosophila* JAK–STAT pathway are Unpaired, an activating ligand [3], and the proteins encoded by a single JAK-like gene, *hopscotch*, and a single STAT-like gene, *dSTAT92E/marelle* (see Figure 1 and [5] for a recent review). The *unpaired* gene, previously known as *outstretched*, encodes a secreted glycoprotein that has been shown to activate downstream components of the JAK–STAT pathway, both genetically [7] and biochemically by the induction of JAK phosphorylation [3].

The determination of sexual identity in *Drosophila* is controlled by expression of the regulator gene *sex-lethal*, which encodes an RNA-binding protein. The initial expression of this gene is driven by the *sex-lethal* establishment promoter Sxl_{Pe}, which is stimulated by positively acting factors encoded on the X chromosome and negatively regulated by the product of the autosomal gene *deadpan*. In embryos destined to be males, the dose of positive regulators encoded by the single X chromosome is insufficient to overcome repression by the product of two autosomal *deadpan* loci, and *sex-lethal* is not expressed (Figure 2). Female embryos, however, contain twice as many copies of the activator genes, present on their two

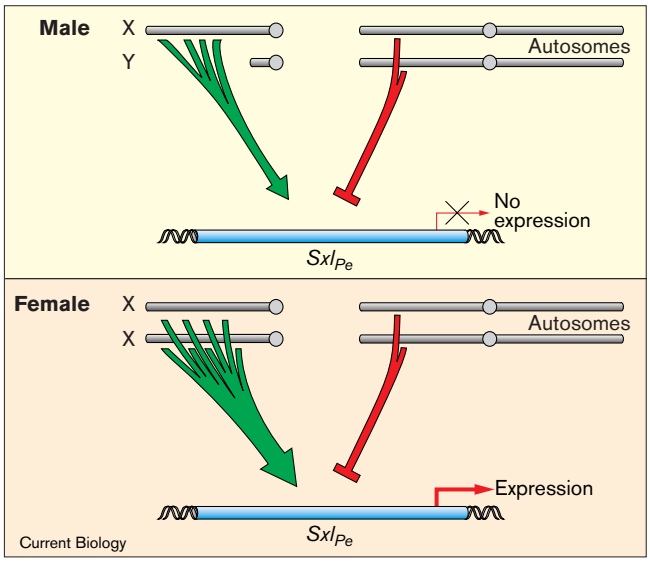
X chromosomes. The extra doses of these activators can overcome the autosomal repression and thus drive *sex-lethal* expression from Sxl_{Pe} (Figure 2). Activation of *sex-lethal* expression in XX embryos is subsequently maintained by a positive autoregulatory feedback loop, leading to development of a female fly (reviewed in [8]).

Given the importance of the activating genes encoded on the X chromosome for the process of sex determination, considerable effort has been made to identify and clone them. The X-linked genes *sisterless-A*, *scute* (previously known as *sisterless-B*) and *runt* have all been shown to encode Sxl_{Pe}-activating transcription factors (see Table 1; reviewed in [9]). In addition to their roles as X-linked activators of *sex-lethal*, most of these genes have other functions during development (Table 1). Moreover, while some of these extra functions are conserved in evolution, it seems that their role in sex determination is more specific to *Drosophila* and may represent relatively recent 're-use' of previously existing molecules and pathways in this process [10].

Because of the essentially additive nature of the X-linked activators, duplications of one or more of these genes results in inappropriate activation of *sex-lethal* expression and male lethality. This characteristic of the sex-determination system prompted a beautifully elegant screen for second-site modifier mutations that result in male progeny from such a duplicated stock. In this way, a new X-linked activator mutation, termed *sisterless-C*, was identified (Table 1 and [1]). The cloning of genes carrying the viable mutations identified by such a screen proved technically challenging, but two recent papers [1,2] nevertheless report the molecular characterisation of *sisterless-C*. This gene turns out to be allelic to *unpaired*, thereby implicating the *Drosophila* JAK–STAT pathway in another developmental process.

In order to visualise the level of X-linked activator activity *in vivo*, a reporter gene expressed only in female embryos and consisting of *lacZ* fused to the Sxl_{Pe} promoter was constructed [1]. As would be expected for a gene encoding a *bona fide* X-linked activator, females that lack *unpaired* function or are homozygous for otherwise viable

Figure 2



A cartoon showing the activators and repressors acting on the *sex-lethal* establishment promoter Sxl_{Pe} in males (top) and females (bottom). In future males, the activators (green arrows) present on the single X chromosome cannot overcome the repression of the two copies of the repressor (red bar) present on the autosome and the Sxl_{Pe} is not activated. In future females, the double dose of activators on the two X chromosomes are sufficient to overcome the repression of the autosomal factors and Sxl_{Pe} is activated.

sisterless-C alleles showed reduced levels of reporter gene expression. Furthermore, embryos lacking *hopscotch* or *dSTAT92E*, which encode downstream components of the JAK–STAT pathway, also showed a reduced level of Sxl_{Pe}:*lacZ* expression, implying that Unpaired also functions upstream of JAK–STAT signalling in this system. In support of this inference, analysis of the Sxl_{Pe} promoter sequence in a region upstream of the transcription start site revealed putative dSTAT92E-binding sites. The complete Unpaired–JAK–STAT signalling pathway thus appears to be involved in activating *sex-lethal* expression.

One aspect of the role of *unpaired* in sex determination is the comparative weakness of the pathway's effect on Sxl_{Pe}.

Table 1

X-linked activators of *sex-lethal* expression.

Gene	Alternative name	Protein	Other functions
sisterless-A	–	bZip transcription factor	–
scute	*sisterless-B*	bHLH transcription factor	Sensory organ precursor development
runt	–	AML1-like transcription factor	Embryo segmentation
unpaired	*sisterless*-C; *outstretched*	Extracellular glycoprotein	Segmentation; haematopoiesis; ommatidial rotation

Genes and their common alternative names are given, as is the nature of the proteins they encode and other developmental events they are involved in. bHLH, basic helix–loop–helix protein; bZip, basic leucine zipper protein; AML, acute myeloid leukemia.

While mutations in *sisterless-A* and *scute* strongly downregulate Sxl_{Pe}*:lacZ* expression and Sex-lethal protein production, removal of Unpaired or its downstream components has a significantly weaker effect, and residual Sxl_{Pe}*:lacZ* expression is still seen in most mutant female embryos [1,2]. For this reason, it was suggested [2] that Unpaired may play a secondary role in the process of X-chromosome counting. Interestingly, such a 'supportive' role for the JAK–STAT pathway has also been suggested in its guise as a regulator of *even-skipped* expression during embryonic segmentation, where residual *even-skipped* expression is detectable even in embryos totally lacking the JAK–STAT pathway [5,11]. This observation led to the suggestion that JAK–STAT signalling is a way of potentiating the activity of other instructive signals.

Another observation relating to the activation of *sex-lethal* by the Unpaired–JAK–STAT pathway is also of interest. Although *sisterless-A* and *scute* activate *sex-lethal* expression throughout the embryo, *runt* and *unpaired* activities are more spatially limited [2]. Runt appears to act only in the central regions of the embryo, and Unpaired acts in a similar region, as indicated both by *sex-lethal* reporter gene activity [2] and the zygotic expression pattern of the *unpaired* gene [3]. While the biological significance of this spatial restriction is unclear, it is intriguing that previous findings [6] indicate that *runt* expression is positively regulated by the JAK–STAT pathway during segmentation. It will be interesting to determine whether the sex-determination activity of Unpaired is mediated in part through Runt.

Finally, the use of an extracellular diffusible ligand such as Unpaired as a component of a cellular autonomous system that counts the X:autosome ratio is also unexpected. All other X-linked activators identified encode DNA-binding transcription factors that act directly on the Sxl_{Pe} promoter; Unpaired, however, relies on maternally deposited Hopscotch and dSTAT92E to transduce its signal. Just why Unpaired has been co-opted as an X-linked activator is not clear, but it is appealing to speculate that the diffusion of extracellular Unpaired may act to 'smooth out' minor differences in cellular response to the autonomously-acting activators. Alternatively, the JAK–STAT pathway may act as a non-linear amplification step so that having two copies of *unpaired*, rather than one, makes a greater than two-fold difference to the level of dSTAT92E-induced promoter activation. In this case, it is possible that the use of Unpaired as an X-linked activator may serve to increase the fidelity of sex determination.

The identification of *sisterless-C* as *unpaired* adds another developmental role — sex determination — to the known functions of the JAK–STAT pathway in *Drosophila* segmentation and haematopoiesis. With *sex-lethal* being only the third target gene identified for the *Drosophila* JAK–STAT pathway, the new results increase our understanding of both sex determination and this important signalling cascade.

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