Plasma Phys. Controlled Fusion **2**, 85 (1975).
77. F. Salin, J. Squier, G. Mourou, G. Vallancourt, *Opt. Lett.* **16**, 1964 (1991); T. B. Norris, *ibid.* **17**, 1009 (1992).
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Sex Determination and Dosage Compensation: Lessons from Flies and Worms

Susan M. Parkhurst and Philip M. Meneely

In both *Drosophila melanogaster* and *Caenorhabditis elegans* somatic sex determination, germline sex determination, and dosage compensation are controlled by means of a chromosomal signal known as the X:A ratio. A variety of mechanisms are used for establishing and implementing the chromosomal signal, and these do not appear to be similar in the two species. Instead, the study of sex determination and dosage compensation is providing more general lessons about different types of signaling pathways used to control alternative developmental states of cells and organisms.

Sex determination is a particularly appealing model system in which to study developmental biology because the developmental decision is made between only two alternative states, male or female, each of which is viable and easily identifiable even within a single cell or a small group of cells. The sum of these individual cellular decisions in the soma, referred to as somatic sex determination, is seen in the whole organism as the morphological differences between male and female. There are also differences in germline sex determination that give rise to either sperm or oocytes and, in levels of X-linked gene expression, a process called dosage compensation.

Although other signals, including environmental cues, are used as a signal in some organisms, the signal for sex determination and dosage compensation in *Caenorhabditis elegans*, *Drosophila melanogaster*, and in mammals is chromosomal. The overall strategy for mammalian sex determination appears to be relatively simple: the phylogenetically conserved *Tdy* (testes-determining on Y) locus, identified on the Y chromosome, acts as a positive signal for testes development and subsequent male sexual differentiation (*1*, *2*). In an apparently independent event, the organism recognizes the number of X chromosomes and inactivates all but one in order to achieve dosage compensation in females (*3*).

In contrast to mammals, the chromosomal signal for sex determination and dosage compensation in *Drosophila* and *C. elegans* is considerably more elaborate. The Y chromosome is not an important signal in *Drosophila* in which males are XY and is not even present in *C. elegans* in which males are normally X0. Instead, the chromosomal signal is the ratio of the number of X chromosomes to the number of sets of autosomes, referred to as the X:A ratio (*4*). Normal animals are diploid, that is, have two sets of autosomes (A = 2). Because females have two X chromosomes, their X:A ratio is 1, whereas males with one X chromosome have an X:A ratio of 0.5. The signal from the X:A ratio is implemented by a hierarchy of a small number of genes that function to control somatic sex differentiation, germline sex determination, or X-chromosome dosage compensation. In recent years, molecular and genetic studies have increased our understanding of the means by which the signal from the X:A ratio is established, maintained, and transmitted to downstream effector genes. In this article, we summarize recent progress in sex determination and dosage compensation in *C. elegans* and *Drosophila* and compare sex determination and dosage compensation in these organisms to these processes in mice and humans.

The authors are with the Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98104, USA.

Somatic Sex Determination

One of the first tasks of a fly embryo is to assess its chromosomal constitution. Transduction of this X:A signal activates a key regulatory gene, *Sex-lethal* (*Sxl*), that in turn regulates the choice between the female and male developmental pathways (*5*). The sex determination decision is cell-autonomous: the decision is stably and independently made by single cells. *Sxl* also regulates dosage compensation to increase X-linked gene expression in males that have only one X chromosome to the same level as that of females that have two X chromosomes. Intermediate X:A ratios yield intersex flies whose tissues are mosaics of male and female cells. Females require continuous *Sxl* activity for proper sexual development, whereas males do not require *Sxl*. Both the lack of *Sxl* expression in females or the constitutive expression of *Sxl* in males results in sex-specific lethality because of inappropriate X-chromosome dosage compensation.

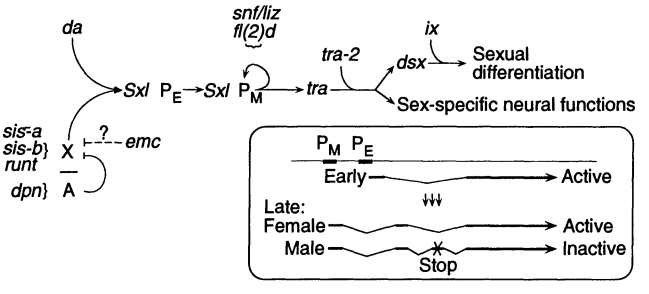

Fig. 1. Summary of the genes and their interactions governing somatic sex determination in *Drosophila*. The primary signal for sex determination is the X:A ratio. Three genes that assess the X component of this ratio (*sis-a*, *sis-b*, and *runt*) work in conjunction with the Da protein to initiate *Sxl* expression at its early promoter, *Sxl* P_E. Two negatively acting factors, one assessing the autosomal component of the X:A ratio, *dpn*, and a maternally required gene, *emc*, inhibit the activity of the X counting elements. Once *Sxl* expression is initiated, a positive autoregulatory feedback loop is established through its late, maintenance promoter, *Sxl* P_M. The products of the *snf/liz* and *fl(2)d* genes appear to be involved in setting up this autoregulatory pathway. Active SXL protein then directs a cascade of sex-specific splicing interactions that culminates in the transcriptional regulation of target differentiation genes that specify male or female morphogenetic products. The inset shows the relation of the *Sxl* P_E and *Sxl* P_M promoters. (Only the splicing for relevant exons is shown.) Early transcripts from the *Sxl* P_E promoter are produced in animals with a high X:A ratio. These transcripts are spliced to yield full-length active SXL protein that in turn is needed for productive splicing of the late transcript in females. Because males do not accumulate full-length active SXL protein from the *Sxl* P_E promoter, they do not exclude an exon containing a stop codon and produce truncated, inactive protein. It is not known how the early transcripts from the *Sxl* P_E promoter are spliced productively in the initial absence of active SXL protein.

Sxl encodes an RNA binding protein (SXL) that regulates sex-specific RNA splicing of its own RNA, as well as the splicing of at least one downstream gene (6). The *Sxl* gene has separate promoters that are distinctly regulated and that establish or maintain *Sxl* activity (7, 8). Initiation of *Sxl* activity is controlled at the level of transcription through an early embryonic promoter, *Sxl* P_E (Fig. 1). This early promoter is stably activated only in animals with a high X:A ratio and is only activated transiently before cellular blastoderm formation. Processed transcripts from the *Sxl* P_E promoter encode full-length active SXL protein. Because this promoter is only active in animals with a high X:A ratio, only embryos with two X chromosomes accumulate active SXL protein.

In contrast to the initiation of *Sxl* activity which is regulated by transcriptional control of the *Sxl* P_E promoter, the maintenance of *Sxl* activity is regulated posttranscriptionally through the maintenance or late promoter, *Sxl* P_M, which is regulated at the level of RNA splicing (6–9). Both sexes begin transcription of *Sxl* from *Sxl* P_M at the cellular blastoderm stage, the time at which the *Sxl* P_E promoter is turning off (8). The transcripts from this late promoter are alternatively spliced to either include or exclude an exon that contains an in-frame translational stop codon and therefore encodes a truncated inactive protein. Removal of this exon requires *Sxl* activity. Because females accumulate active SXL protein derived from the early promoter, they splice the late transcript to make full-length active SXL protein. Thus, a positive autoregulatory

Table 1. Genes involved in *Drosophila* somatic sex determination. Blank spaces indicate unknown or unpublished data; aa, amino acid.

Gene	Phenotype	Product and homology	Regulation and function	References
Sxl	Loss-of-function: XX: masculinized, lethal XY: no effect	Multiple sex-specific RNAs. Active protein products have homology to RNA binding proteins.	Embryo-specific promoter, P_E, activates *Sxl* in response to high X:A ratio.	*5–9*
	Gain-of-function: XX: no effect XY: feminized, lethal		Non–sex-specific promoter, P_M, produces alternatively spliced male and female transcripts. Female protein active and required for splicing of its own transcripts and those of *tra*. Male protein truncated and inactive.	
sis-b	Zygotic XX: embryonic lethal XY: no effect	bHLH transcriptional activator	Numerator. Functions in all somatic nuclei to activate *Sxl* transcription.	*13, 14*
sis-a	Zygotic XX: embryonic lethal XY: no effect	bZIP transcriptional activator	Numerator. Functions in all somatic nuclei to activate *Sxl* transcription.	*11, 12*
runt	Zygotic XX: embryonic lethal XY: no effect	Runt domain (heterodimeric transcription factor)	Numerator. Weaker than *sis* genes. Activates *Sxl* transcription in a spatially restricted domain in female embryos.	*16, 17*
da	Maternal effect XX: embryonic lethal XY: no effect	bHLH transcription factor	Maternally supplied activity required in female embryos as a positive activator of *Sxl*.	*19*
dpn	Zygotic Reduced male viability	bHLH transcriptional repressor	Denominator. Inhibits *Sxl* activation in males.	*18*
emc	Maternal effect Reduced male viability	HLH transcriptional repressor	Maternal activity inhibits *Sxl* activation in males.	*21*
snf or *liz*	No effect in presence of two Sxl^+ genes. Transheterozygous with *Sxl* mutation leads to somatic sex transformation and reduced viability.		Maternal effect. Positive regulator of *Sxl* functions in females. Affects late, but not early, female-specific spliced *Sxl* RNAs and proteins. Possibly involved in *Sxl* autoregulatory feedback loop.	*50*
fl(2)d	XX: lethal XY: semi- to fully lethal		Interacts with *Sxl*. Mutant females express male-spliced *Sxl* transcripts. Possibly involved in *Sxl* autoregulatory feedback loop.	*51*
tra	XX: pseudomales XY: no effect	Two classes of transcripts that differ in 3′ splice acceptor after first intron: 0.9 kb female-specific, functional; 1.1 kb non–sex-specific, nonfunctional.	Required in conjunction with *tra-2* to regulate *dsx* in females. Inactive in males. Requires active *Sxl* protein for its proper splicing.	*5, 29*
tra-2	XX: pseudomales XY: males, but sterile	Multiple transcripts and proteins. Homology to RNA binding proteins.	Required in conjunction with *tra* to regulate *dsx* in females. Inactive in males.	*5, 29*
dsx	Null alleles transform XX and XY to sterile intersexes. Some alleles affect only one sex.	Several sex-specific RNAs via alternative splicing yielding 427-aa female-specific and 549-aa male-specific protein products.	Bifunctional. Female product represses male differentiation in females. Male product represses female differentiation in males.	*5, 29*
ix	XX: intersex XY: intersex		Required in conjunction with *dsx* to repress male differentiation.	*5, 29*

feedback loop at the level of RNA splicing is established, which allows females to stably maintain *Sxl* in an ON state. Because males do not accumulate active SXL protein from the *Sxl* P_E promoter during the short time it is active, they cannot exclude the exon with the stop codon or activate productive *Sxl* splicing from either promoter. The truncated protein they produce is inactive in splicing, hence males stably maintain *Sxl* in an OFF state. Consistent with their requirement for *Sxl*, females contain high amounts of full-length SXL protein from the blastoderm stage onwards, whereas males have none (*7*).

A number of loci have been implicated in the activation of *Sxl* but not in directly reading the X:A ratio. These are summarized in Table 1, but additional work is needed to establish their exact role in regulating *Sxl*.

X:A signaling in flies. In the soma, the X:A ratio is assessed by means of dispersed chromosomal genes termed counting elements; X-chromosomal genes are referred to as numerators, and the autosomal genes are referred to as denominators (*4*). Numerators behave as feminizing elements because they increase the probability of activating *Sxl* expression from the *Sxl* P_E promoter. Reducing the dosage of numerator genes leads to female lethality because of failed *Sxl* expression, whereas increasing the dosage of such genes activates *Sxl* inappropriately, leading to male lethality. The lethality is not a consequence of choosing the wrong sex but rather is a consequence of inappropriate dosage compensation. Denominators, on the other hand, behave as autosomal masculinizing genes that antagonize feminizing numerators. Significant insight has been gained into the mechanism of X:A signal trandsuction through the molecular cloning and characterization of several counting elements. The properties and phenotypes of genes involved in fly somatic sex determination are summarized in Table 1. A number of these genes are also utilized in neural development, suggesting some common regulatory mechanisms exist for the two processes.

Three genes have been identified in flies that fit the criteria for numerators: *sisterless-a* (*sis-a*), *sisterless-b* (*sis-b*), and *runt*. A weaker fourth numerator, *sis-c*, has also been identified, but its properties have not yet been published (*10*). The *sis-a* gene was the first female-specific X-linked mutation to be identified that functions as a positive regulator of *Sxl* (*11, 12*). The *sis-a* gene encodes a protein with homology to the basic-leucine zipper (bZIP) family of transcriptional regulators (*10*). A second numerator locus, *sis-b*, was identified by virtue of its genetic interaction with *sis-a*. Simultaneous duplication of *sis-b*$^+$ and *sis-a*$^+$ activates *Sxl*$^+$ activity in males (*12*), causing male lethality as a result of disruption of dosage compensation. The *sis-b* gene corresponds to the *scute-α/T4* gene, one of several genes that encode basic-helix-loop-helix (bHLH) proteins within the *achaete-scute* complex (AS-C). Mutations in the *T4* gene affect sex determination, and P-element transformation of a wild-type *T4* gene rescues *sis-b* mutations. In addition, *T4* overexpression activates *Sxl* expression in male embryos (*13–15*). We refer to the *sis-b/scute-α/T4* gene as *sis-b* in this review in recognition of its sex determination activity. Both *sis* genes are expressed transiently throughout the embryo during the early syncytial stages before the initiation of expression from the *Sxl* P_E promoter, and these genes activate *Sxl* transcription from this promoter in all somatic cells. There is some redundancy in these loci because duplications of *sis-b*$^+$ partially rescue *sis-a* mutations (*12, 14, 15*).

A third numerator locus, *runt*, has weaker effects on chromosome counting than the *sis* genes (*16*). Unlike the *sis* genes, *runt* only affects sex determination in the central trunk region of the embryo. This gene encodes a nuclear protein that shares homology with a family of transcriptional regulators, which includes the α subunit of the heterodimeric polyoma enhancer binding protein (PEBP2) and the human acute myeloid leukemia gene *AML1*. The homology of *runt* and PEBP2 lies in regions of PEBP2α that are responsible for DNA binding and dimerization (*17*). This suggests that *runt* may also form protein heterodimers or bind to DNA or both.

Bridges postulated the existence of denominator elements because flies with two X chromosomes and three sets of autosomes (2X:3A = 0.67) develop as intersexes rather than as females (*4*). Only one candidate for a denominator has been identified to date, the neural gene *deadpan* (*dpn*). It encodes a negatively acting HLH protein that behaves as a dosage-sensitive masculinizing element required for inhibiting *Sxl* activation in males (*18*). It seems likely that other genes fulfilling the criteria for denominator elements exist and remain to be found.

In addition to the numerator and denominator elements described, which encode proteins expressed in the zygote, both positively and negatively acting maternal factors are required for the transduction of the X:A signal and the establishment of *Sxl* expression. The neural gene *daughterless* (*da*), a bHLH transcription factor, is required maternally to activate *Sxl* (*19*). Female progeny of *da* mothers die, whereas male offspring survive because they do not require *Sxl*. HLH proteins work as heterodimers, and the Da protein interacts with other bHLH proteins, in particular, with Sis-b. Consistent with this, Da–Sis-b protein heterodimers bind DNA in vitro and also activate transcription in yeast (*20*).

The maternal product of another neural gene *extramacrochaetae* (*emc*) can act as a negative regulator of *Sxl* (*18*). The Emc protein contains an HLH motif needed for protein dimerization but lacks the conserved adjacent basic residues required for DNA binding (*21*). Such HLH proteins may negatively regulate other bHLH proteins by forming nonfunctional heterodimers that do not bind DNA (*22*).

X:A activation of Sxl. The molecular characterization of the first numerator element, *sis-b*, suggested a model for regulation in which heterodimers between the Da and Sis bHLH proteins activate transcription of *Sxl* from the *Sxl* P_E promoter (*8, 13, 14*). The da RNA is contributed maternally and translated equally in early 1X and 2X embryos. Thus, the likelihood of activating

Table 2. Genes involved in *C. elegans* sex determination and dosage compensation. Blank spaces indicate unknown or unpublished data; aa, amino acid.

Gene	Phenotype	Product and homology	Regulation and function	References
xol-1	XO: lethal, feminized XX: no effect			*26*
sdc-1	XO: no effect XX: masculinized and high X-linked gene expression	1203-aa protein with seven zinc fingers.	maternal, not sex-specific?	*26, 27*
sdc-2	Like *sdc-1* but more severe. Some alleles lethal in XX.			*26*
sdc-3	XO: no effect XX: sex determination and dosage compensation defects genetically separable. Null alleles have no overt sex determination defects.	250-kD protein with two zinc fingers and a cofactor binding motif.	maternal, not sex-specific?	*28*

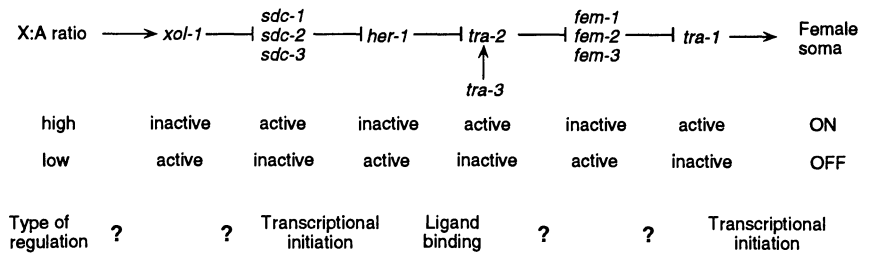

Fig. 2. Summary of the genes and their interactions governing somatic sex determination in *C. elegans*. The pathway regulating somatic sex determination is described by a series of negative regulatory interactions, as depicted by ⊣. The order of the genes in this pathway and the nature of the interactions has been determined by the construction of double and multiple mutant strains [reviewed in (*26*)]. Genes shown together apparently control the same process and may work as a complex, although the nature of their interactions has not yet been solved. Molecular analysis has suggested the type of regulation employed for several steps, although these have not been proved in any case. These postulated types of regulation are shown beneath the interaction.

Sxl transcription must depend on limiting concentrations of Sis proteins that are expressed at twofold higher amounts in 2X embryos. Sis amounts in 1X embryos would not be sufficient to activate the *Sxl* P_E promoter. In such a model, denominator proteins such as Dpn and negatively acting maternal proteins such as Emc are present in equal amounts in male and female embryos. These proteins act by reducing the effective concentration of numerator proteins, possibly by forming heterodimers that cannot activate *Sxl* P_E (Fig. 1).

By extension, the other numerator elements are postulated to be dosage-sensitive activators of the *Sxl* P_E promoter. In agreement with this model, all of the X:A signaling loci characterized to date encode subunits from different families of heterodimeric transcription factors. These proteins may interact with other members of their own family or even with members of other families. For example, Da protein may bind to non-bHLH proteins such as Sis-a and Runt. Interactions have been demonstrated between different families of transcription regulators, for example, between the MyoD bHLH domain and the c-Jun bZIP domain and between the MyoD bHLH domain and the retinoblastoma pocket domain (*23*). There may also be unidentified partners for Sis-a and Runt within their respective families. Because they contain both positive and negative transcriptional regulators, these heterodimeric families may compete with each other or work cooperatively, and these interactions could occur either on or off the DNA. This model assumes that all positive and negative factors act simultaneously to result in *Sxl* being ON or OFF. Alternatively, the denominator elements may act later to ensure that the *Sxl* P_E promoter is not activated in males even when numerator expression peaks (*10*).

Whereas neither numerators nor denominators have been shown to act directly by binding to the *Sxl* P_E promoter, expression of this promoter is the first difference seen between males and females, so it seems likely that this is their mode of action. An immediate task is to determine the interactions of these various transcriptional regulators with each other and their role in transducing the X:A signal to the *Sxl* P_E promoter.

X:A signaling in worms. Although there are genes with functional similarities, no unifying gene like *Sxl* has been identified in *C. elegans*, and some have questioned whether such a gene exists (*24*). In addition, true numerators, that is, X-linked loci with dosage-sensitive effects on sex determination and dosage compensation in diploids, have not been reported in *C. elegans*, although about 20% of the X chromosome remains to be tested for numerator activity (*25*). Nevertheless, work with polyploid animals has shown that there is an X:A ratio, and four genes that respond to it to control both dosage compensation and sex determination have been identified (*26*). The phenotypes and properties of these genes are summarized in Table 2. Three of the four genes, *xol-1*, *sdc-1*, and *sdc-2*, are X-linked. However, none of these genes is normally dosage-sensitive, at least at the physiological levels of one functional copy or two. In addition, wild-type *xol-1* activity is needed for male, but not for female, development. Therefore, these genes are unlikely to be numerators. The *sdc-1* gene encodes a protein with zinc fingers and is probably involved in regulating transcription (*27*). The molecular characterization of the other two X-linked genes, *xol-1* and *sdc-2*, has not yet been reported.

The fourth gene, *sdc-3*, is autosomal and encodes a protein with two zinc fingers near the COOH-terminus (*28*). Mutations in the zinc fingers result in dosage compensation defects, whereas mutations with sex determination defects map to a different region of the protein that has limited homology to cofactor binding domains. One explanation of the means by which *sdc-3* regulates both sex determination and dosage compensation is that the DNA binding specificity of the zinc fingers depends on the presence or absence of a sex-specific cofactor. In this hypothesis, the sex-specific cofactor could simply be the product of an X-linked gene that is present at higher amounts in XX animals than in X0 animals, a protein functionally equivalent to *Drosophila* numerators.

Transduction of the X:A signal to downstream effector genes. The products of the genes that respond to the X:A ratio suggest the means by which this signal is transmitted to downstream effector genes. The SXL protein is an RNA binding protein that controls the splicing of its own messenger RNA (mRNA) and that of the downstream gene *tra* that is involved in somatic sex determination. The entire *Drosophila* pathway downstream of *Sxl* that controls somatic sex determination is regulated by alternative splicing (Fig. 1). The pathway branches after the *tra* and *tra-2* genes and ends with specific terminal regulators (*29*). For somatic sexual differentiation, the cascade ends with *doublesex* (*dsx*), a bifunctional gene that regulates the transcription of sex-specific effector genes such as the yolk proteins in females. Readers are referred to current reviews that present this splicing cascade in depth (*5*, *29*, *30*).

Although the mutant phenotypes of the genes that control somatic sex determination in *C. elegans* are superficially similar to those in *Drosophila*, molecular analysis of these genes has demonstrated that the regulation is probably accomplished by a pathway combining both transcriptional regulation and ligand-receptor interactions. The properties of seven genes implicated in somatic sex determination are summarized (Table 3 and Fig. 2). At the top of this group of genes is *her-1*, whose expression is transcriptionally regulated, presumably by the *sdc* genes (*31*). The functional Her-1 gene product is likely to be a secreted protein and requires an NH_2-terminal signal sequence to specify male development (*24*). The *her-1* gene may encode the ligand for a receptor encoded by *tra-2*, because the major *tra-2* product is predicted to be an integral membrane protein (*32*). Thus, the putative Her-1 ligand is made and secreted in X0 animals in which it binds to the Tra-2 receptor and turns off *tra-2* function (*24*, *32*). This extracellular effect of *her-1* is consistent with the genetic demonstration that *her-1* is not cell-autonomous (*33*). The cellular nonautonomy of *her-1* function is very different from the cell autonomy of the somatic sex determination process in *Drosophila*. Whereas this model of somatic sex determination in *C. elegans* is certainly plau-

sible on the basis of sequence comparisons, many aspects remain to be demonstrated, such as membrane localization of the Tra-2 protein or a direct physical interaction between the *her-1* and *tra-2* gene products. If Tra-2 protein is membrane-bound, its activity must be relayed from the cell surface to the nucleus. The activity of the Tra-2 protein is negatively regulated through three *fem* genes, but their molecular analysis has not yet elucidated the means by which this regulation is achieved (*34*).

The final regulatory gene in the somatic sex determination pathway in *C. elegans* is the autosomal gene *tra-1* (*35*). When *tra-1* is active, hermaphrodite (female) development occurs; when *tra-1* is inactive, male development occurs. The *tra-1* gene produces two transcripts, the larger of which is predicted to encode a protein, Tra-1L, with five zinc fingers that is clearly essential for *tra-1* activity (*36*). The smaller transcript terminates after the first two zinc fingers; its product is called Tra-1S. The zinc fingers of Tra-1L bind to DNA in vitro, whereas the zinc fingers of Tra-1S do not (*37*). Furthermore, removal of the first two zinc fingers from Tra-1L does not affect its ability to bind DNA. Therefore, Tra-1S may act as a bodyguard and titrate negative regulators of Tra-1L, for example, the products of the *fem* genes, thereby enhancing Tra-1L expression (*36, 37*). Because the final gene in the pathway for somatic sex determination is a DNA binding protein that would be active when the X:A ratio is high, it is appealing to imagine that Tra-1L is a transcription factor for downstream female-specific genes. However, the targets of Tra-1L have not yet been identified.

Thus, the final gene in the regulatory cascade in both flies and worms is a DNA binding protein and is likely to be a transcription factor. Ten years ago it would not have been naïve to predict that the entire regulation of somatic sex determination was accomplished by means of a series of transcription factors. Therefore, one of the more surprising conclusions from the molecular analysis of this process is that few steps are controlled at the initiation of sex-specific transcription. In most cases, the genes are transcribed in both sexes but produce sex-specific protein products or protein products functional in only one sex, indicating that their functions are controlled by posttranscriptional processes.

Germline Sex Determination

Drosophila. The precursors of the *Drosophila* germline are the first cells formed in the embryo. These cells, known as pole cells, form at the posterior end of the preblastoderm embryo. At this early stage, they can be transplanted from donor embryos into host embryos in which they will later be integrated into the developing gonad. Such germ cell transplantation experiments have shown that like their somatic counterparts, *Drosophila* germ cells require *Sxl* activity to undergo female development (Fig. 3) (*38, 39*). However, although both somatic and germ cells require the cell autonomous X:A signal, female (2X:2A) germ cells also require inductive signals from the surrounding soma to activate *Sxl*: The 2X:2A germ cells develop according to the sex of the surrounding soma, whereas the 1X:2A germ cells enter the male developmental pathway independent of the sex of the surrounding somatic cells (*40*). The influence of the surrounding soma on female germ cells suggests that inductive signals are required from the soma, whereas the ability of XY or X0 germ cells to undergo spermatogenesis regardless of the sex of the surrounding soma provides evidence for a cell autonomous X:A signal (*41*).

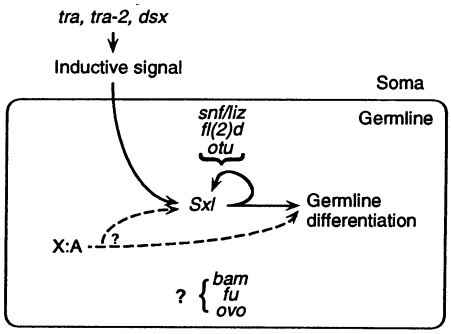

Fig. 3. Summary of germline sex determination in *Drosophila* females. Sex determination in the germline (box) requires both the cell-autonomous X:A signal and an inductive signal from the soma. It is not yet clear if the target of the X:A signal is the *Sxl* gene (dashed line). However, *Sxl* is the target of the inductive signal. The downstream regulatory genes from the somatic sex determination pathway (*tra, tra-2,* and *dsx*) are required in the soma for the production of the inductive signal. Target genes downstream of *Sxl* have yet to be identified. Several genes have been identified that are needed in this pathway, although their exact roles have yet to be defined. Three of these (*snf/liz, fl(2)d,* and *otu*) may be involved in the establishment of the *Sxl* positive autoregulatory feedback loop.

Table 3. Genes involved in *C. elegans* sex determination, but not dosage compensation. Blank spaces indicate unknown or unpublished data; aa, amino acid.

Gene	Phenotype	Product and homology	Regulation and function	References
her-1	Recessive alleles cause XO animals to become fertile hermaphrodites. Dominant alleles masculinize XX.	Two transcripts. Larger encodes a secreted protein of 175 aa.	Male-specific transcription. Possible ligand?	*24, 31*
tra-2	Recessive alleles transform XX into pseudomales, with no effect on XO. Dominant alleles affect germline.	Three transcripts. Largest protein likely membrane bound protein of 1475 aa.	Possible sex differences in transcript abundance. Possible receptor?	*32, 59*
tra-3	Maternal effect. Recessive alleles like weak *tra-2* recessive alleles.		Maternal activator of *tra-2* function?	*26*
fem-1	XO: females XX: females	Protein of 656 aa with six ankyrin repeats.	No sex differences in transcript levels. Maternal.	*34*
fem-2	XO: females XX: females		Maternal.	*26*
fem-3	Recessive alleles like *fem-1* and *fem-2*. Dominant alleles masculinize germline.	Protein of 388 aa.	Maternal, regulated posttranscriptionally.	*34, 58*
tra-1	Recessive alleles transform XX into mating males, minor effects on gonad in XO. Dominant alleles feminize XO and XX. Genetically complex.	Two proteins. Tra-1S has two zinc fingers, Tra-1L has five zinc fingers. Homology to human GL1 and GL3.	Large protein binds DNA. Small protein does not; role in autoregulation?	*35–37*

Inductive signal. The inductive signal that emanates from the soma to XX germ cells is established during embryogenesis after somatic sex determination has taken place. This signal is dependent on the downstream sex determination functions including *tra*, *tra-2*, and *dsx* in the soma and appears to regulate the expression of *Sxl* in the germline (*39*, *40*, *42–45*). The inductive signal from somatic cells could regulate *Sxl* in either of two ways: XX germ cells undergo spermatogenesis unless they receive a feminizing signal from the female soma that leads to *Sxl* activation, or XX germ cells undergo oogenesis unless they receive a masculinizing signal from the male soma that leads to the repression of *Sxl* (*40*, *45*).

Cell-autonomous X:A signal. Although both the X:A signal and *Sxl* activity are required for sex determination in the germline, the mechanism by which *Sxl* is activated is different from that in somatic cells. In the soma, the X:A signal is transduced by a number of transcription factors that lead to the activation of *Sxl* expression at the early *Sxl* P_E promoter. Female germ cells, like their somatic counterpart, require sex-specific processing of their *Sxl* transcripts and a positive autoregulatory feedback loop to maintain productive *Sxl* splicing (*44*, *46*, *47*). The SXL protein needed for this splice in the soma comes from the *Sxl* P_E promoter. However, this promoter is not active in germ cells (*8*), and it is unknown how productive *Sxl* splicing is initially achieved in germ cells.

Consistent with the lack of activity of *Sxl* P_E, *sis-a*, a component of the somatic X:A signaling system, is not required for oogenesis (*10*). In addition, *Sxl* is not activated in somatic cells mutant for the *sis-b* gene or simultaneously heterozygous for *sis-b*, *sis-a*, and *runt* (a condition that is more severe than homozygosity for the individual mutations), yet germ cells of these genotypes produce functional oocytes when transplanted into female hosts (*45*, *48*). Together, these results suggest that *da*, *sis-a*, *sis-b*, and *runt* are not required for germline sex determination.

The targets of *Sxl* regulation are different in the germline and soma because *tra*, *tra-2*, *dsx*, and *ix*, target genes identified in the somatic pathway and needed for the somatic inductive signal to the germline, are dispensible in the germ cells themselves (*43*). However, no targets of *Sxl* specific to the germline have been identified.

Despite the fact that the known somatic regulators and targets of *Sxl* are not involved, it is presumed that *Sxl* is the target of the X:A ratio in the germline. However, constitutive expression of *Sxl* in the germline with the use of a strong constitutive mutant of *Sxl*, Sxl^{M4}, does not drive XY germ cells into oogenesis (*48*). Two interpretations have been put forward to explain this observation: Either the Sxl^{M4} allele is not constitutive for all aspects of *Sxl* function (that is, it does not express *Sxl* functions in XY germ cells), or *Sxl* expression is not sufficient for germ cells to undergo oogenesis. Further experiments are needed to distinguish between these possibilities and determine if the X:A signal works in parallel or synergy with the inductive signal to initiate germline sexual differentiation.

Although the majority of somatic sex determining genes are not required in germ cells, several genes involved in germline sex have been identified (Table 4). These genes are members of the ovarian tumor group and fall into several phenotypic classes (*41*, *44*). One class, including the genes *sans fille* or *liz* (*snf/liz*), *fl(2)d*, and some alleles of the *ovarian tumor* (*otu*) locus, form ovaries with multicellular cysts, a phenotype similar to that caused by *Sxl* germline-specific mutations (*49–51*). In the wild-type ovary, Sxl RNA and protein are distributed in highly dynamic patterns. In addition to the spatial and temporal regulation of the protein, dramatic changes in SXL subcellular localization also occur. In ovaries from mutants of the ovarian tumor group of genes, no SXL protein is detected, and mutant ovaries contain high amounts of Sxl RNA spliced in the male mode. Thus, this class of genes may be needed for *Sxl* autoregulation (*44*, *47*).

A second class of genes involved in germline sex determination includes *bag-of-marbles* (*bam*) and *fused* (*fu*). Analysis of the *bam* mutation has suggested that it is involved in early cystocyte divisions, whereas the *fu* mutant encodes a serine-threonine kinase implicated in cell-cell interactions (*52*, *53*). *Sxl* splicing is predominately in the

Table 4. Genes involved in *Drosophila* germline sex determination. Blank spaces indicate unknown or unpublished data; aa, amino acid.

Gene	Phenotype	Product and homology	Regulation and function	References
Sxl	Tumorous ovary (ovaries filled with cysts of small undifferentiated cells)	RNA binding protein with two RNP domains	Requires X:A signal and inductive signals from soma. Germline-specific transcripts. No downstream target genes identified to date.	*46*, *47*
snf or *liz*	Tumorous ovary		Active SXL protein expression reduced. Mutant ovaries exhibit high amounts of male-spliced *Sxl* RNA. Possibly required for *Sxl* autoregulation.	*44*, *47*, *50*
otu	Tumorous ovary	Cytoplasmic proteins of 98 kD and 104 kD from alternatively spliced RNAs. Weak similarity to *bam* in three regions spanning 150 aa.	Active SXL protein expression reduced. Mutant ovaries exhibit high amounts of male-spliced *Sxl* RNA. Possibly required for *Sxl* autoregulation.	*44*, *47*, *49*
fl(2)d	Tumorous ovary		Mutant ovaries exhibit high amounts of male-spliced *Sxl* RNA. Possibly required for *Sxl* autoregulation.	*51*
bam	Tumorous ovary	Protein of 442 aa with weak similarity to *otu* in three regions spanning 150 aa.	High amounts of SXL protein. SXL intracellular protein distribution altered. Possibly involved in control of cystocyte divisions.	*44*, *47*, *52*
fu	Tumorous ovary	Serine-threonine kinase	High amounts of SXL protein. SXL intracellular protein distribution altered.	*44*, *47*, *53*
ovo	Germ cells absent	At least a 1209-aa protein with four zinc fingers (transcription factor).	Required in XX germ cells from late blastoderm stage onwards. Probably does not act through *Sxl*.	*44*, *54*, *55*

female mode in both of these mutants (47). The tumorous ovaries of *bam* and *fu* mutants have normal amounts of *Sxl* protein, but the protein is not localized properly, leading to the suggestion that this signaling system might mediate communication between the interconnected cells of the cystocyte or between somatic and germ cells (47).

The *ovo* mutation belongs to a third class because, in contrast to the mutations listed above which produce tumorous germlines, *ovo* mutant mothers develop rudimentary germlines that later degenerate and yield no functional eggs (54). The *ovo* gene has been characterized molecularly and encodes a transcription factor with a zinc finger motif (55). A role for *ovo* in dosage compensation of germ cells has been proposed (44). However, additional studies are needed to further define what processes the *ovo* gene affects.

Thus, although *Sxl* is a key regulatory gene for both somatic and germline sex determination, the genes needed in the soma and germline for *Sxl* regulation are different. The means by which these different classes of genes carry out their functions awaits additional genetic and molecular characterization.

Nematodes. There appears to be little similarity between germline development in *Drosophila* and *C. elegans*. Among other differences, XX animals in *C. elegans* are hermaphrodite rather than female. (Hermaphroditism is probably a secondary evolutionary modification of a basic XX female–XO male system). In these animals, the same pool of meiocytes gives rise first to sperm and then to ova (56). Thus, germ cells can follow one of three developmental pathways: oogenesis, hermaphrodite spermatogenesis, or male spermatogenesis. Spermatogenesis in males and hermaphrodites is largely controlled by the same genes, suggesting that hermaphrodites are somatic females that turn on the male spermatogenesis pathway for a brief time before beginning oogenesis.

In contrast to *Drosophila*, all of the genes that affect somatic sex determination in *C. elegans* also affect the germline. The switch between spermatogenesis and oogenesis is accomplished by regulation of the activity of *tra-2* and *fem-3* (Fig. 4) (57). When *tra-2* is inactive, *fem-3* (and the other *fem* genes) is active and spermatogenesis occurs. This is the permanent situation in males and a transient situation in hermaphrodites. Conversely, when *tra-2* is active, *fem-3* is inactive and oogenesis ensues. There are constitutive alleles of these two genes that escape inactivation. Thus, the *tra-2* constitutive mutants produce only oocytes, and the *fem-3* constitutive mutants produce only sperm (57). Although the regulation of these genes might be complex, one means by which both genes are regulated is by translational control which requires specific sequences in the 3′ untranslated regions of their transcripts (58, 59). An activity that binds to a specific sequence in the 3′ untranslated region of the *tra-2* transcript and regulates its expression has been identified (59). This activity is not germline specific, indicating that it may also regulate other genes. This activity does not bind to the fem-3 RNA, so the factor that regulates *fem-3* has yet to be identified.

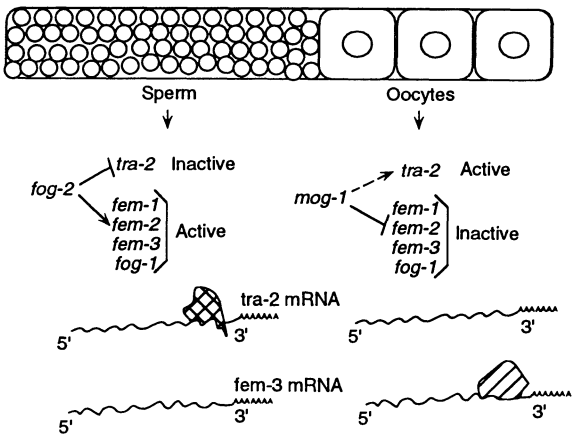

Fig. 4. Summary of germline sex determination in *C. elegans* hermaphrodites. The hermaphrodite makes first sperm and then oocytes within the same gonad from the same pool of meiocytes. The switch from spermatogenesis to oogenesis can be accomplished by regulation of the genes *tra-2* and *fem-3*. Sperm production requires that *tra-2* be inactivated; one means by which this may occur is a translational block in the 3′ untranslated region of the tra-2 mRNA, indicated at the bottom of the figure. The *fem* genes and *fog-1* are active when *tra-2* is inactive. The gene *fog-2* is required for spermatogenesis in the hermaphrodite, either by inactivating *tra-2* or by activating the *fem* genes and *fog-1*. Once sperm are produced and stored, spermatogenesis is shut off and oogenesis begins. It is not clear if *tra-2* is reactivated (59), but the *fem* genes and *fog-1* are functionally inactivated, a process requiring *mog-1*. Inactivation could involve any or several of the *fem* genes and *fog-1*, although only *fem-3* has been shown to be involved, and the inactivation of *fem-3* is sufficient to shut off spermatogenesis. Inactivation of *fem-3* may also be achieved by translational regulation by a protein distinct from the one regulating *tra-2*.

In addition to *tra-2* and the *fem* genes, which affect both somatic and germline sex determination, three other genes (*fog-1*, *fog-2*, and *mog-1*) affect germline sex determination specifically. A fourth gene, *fog-3*, has been identified but its properties have not been published (60). Fog mutants do not produce sperm, whereas the Mog mutant does not produce oocytes. The *fog-1* gene is thought to act in concert with the *fem* genes to establish and maintain spermatogenesis in both sexes (61). In contrast, *fog-2* is likely to be an important and possibly specific activator of hermaphrodite spermatogenesis. The *fog-2* gene appears to be a main regulator of the choice between sperm and oocytes and could function either by inactivating *tra-2* or by activating *fog-1* or the *fem* genes (62). The *mog-1* gene is a candidate for a negative regulator of *fem-3* (63). The negative regulation of *fem-3* need not be direct, because the negative regulation of any of the *fem* genes or of *fog-1* is sufficient to effect the switch from spermatogenesis to oogenesis. Nonetheless, an appealing hypothesis is that *mog-1* directly regulates the sperm to oocyte switch by blockage of the translation of *fem-3* in the germline. A maternal *mog-1* activity is also needed during embryogenesis, although this may well be independent of its effect in the germline (63). The *mog-1* gene may block the translation of a variety of stored mRNAs, such as maternal mRNAs in the embryo and fem-3 (or other) mRNAs in the hermaphrodite germline.

Although germline sex determination and somatic sex determination must correspond, flies and worms differ fundamentally in the means by which this is accomplished. For worms, all of the genes that regulate somatic sex determination also control germline sex determination. Worm germline sex determination also has additional controls, perhaps as a result of the existence of hermaphrodites rather than females. For flies, concordance between germline sex and somatic sex probably arises because the major genes involved in somatic sex determination, namely *tra*, *tra-2*, and *dsx*, establish an inductive signal from the soma to the germline. This signal acts in some way on *Sxl*; however, the control of *Sxl* expression in the germline remains to be clarified.

Dosage Compensation

In addition to controlling both somatic and germline sex determination, the X:A ratio also controls the intimately linked process of dosage compensation (64). Dosage compensation refers to the equalization of X-linked gene expression between 1X and 2X animals. Dosage compensation is widespread among animals and nearly universal among animals with heteromorphic sex chromosomes. However, the means by which dosage compensation is accomplished varies widely from species to species, with the net effect being the compensation for a mere twofold difference in expression. Such a twofold difference is

more subtle than amounts generally analyzed for gene regulation and is hard to measure reliably by most experimental procedures currently in use. Mechanisms that could compensate for a twofold difference might include slight adjustments in the initiation of transcription, the rate of transcriptional elongation, the stability of the mRNA, the transport of the mRNA to the ribosomes, and so on. This compensation could occur in either sex or in both. In fact, although the outcome is the same, flies compensate by increased X-linked gene expression from the single X chromosome of males, whereas nematodes compensate by decreased gene expression from the two X chromosomes of hermaphrodites.

For the X:A ratio to function, it is crucial that the embryo can distinguish between one X chromosome and two X chromosomes. For *Drosophila*, in which the initial steps in the X:A ratio have been described in detail, the dosage-dependence of *sis-b* and probably other numerators is a consequence of being expressed before dosage compensation is established. Although the numerators are unknown in *C. elegans*, it is possible that they too will not be subject to dosage compensation. In flies, once the numerators activate *Sxl*, dosage compensation is established and differences in X-linked gene expression are eliminated.

The maintenance of dosage compensation in flies requires four autosomal genes: *male-specific lethal (msl)-1*, *-2*, and *-3*, and *maleless* (*mle*) (65). These genes are under the control of *Sxl*, but exactly how this control is exercised is not understood because neither of the cloned dosage compensation genes (*msl-1* and *mle*) appears to be regulated by differential splicing (66). The *mle* and *msl-1* genes are expressed in both sexes, yet their protein products associate with the X chromosome only in males (66). This chromosome-specific localization, but not the expression of the genes themselves, is dependent on the function of the other dosage compensation genes and is prevented by functional *Sxl* product (67, 68). Although the significance of the difference is not clear, Mle and Msl-1 appear to differ in their localization in females (66). Mle is found dispersed at a number of consistent but low-level sites on both the autosomes and the X chromosome, whereas Msl-1 does not appear to bind to the polytene chromosomes in females at all. Although *msl-1* encodes a previously uncharacterized protein, the *mle* product is likely to be an RNA helicase. This may indicate an effect on transcriptional elongation or translational initiation, but the exact physiological role of RNA helicases is unclear (69). Given that the absence of any one of the male-specific lethal genes causes a defect in dosage compensation and a failure of Mle to associate with the male X chromosome, a plausible postulate is that the four gene products work together in a chromosomal transcription complex (66, 67). Molecular analysis of other dosage compensation genes may help resolve this, although some of the answers may result from an understanding of X-chromosome structure in males and females.

Both venerable and recent cytological evidence has shown that the X chromosome in many species has distinct cytological properties. For example, the polytene X chromosome in *Drosophila* is more diffuse in males than in females (65). In addition, antibodies that recognize a specific acetylated isoform of histone H4 stain the polytene X chromosome in males but not in females (70). This chromosome-specific staining requires the wild-type products of the male-specific lethal genes, suggesting that the *msl* gene products are responsible for localizing the acteylated histone isoform (68). The acetylated histone may itself result in a chromatin conformation more accessible to transcription or it may serve as a recognition site for other proteins that increase transcription. This observation still does not explain how the *msl* gene products become localized to the X chromosome initially; that is, what molecular properties distinguish the X chromosome from the autosomes in the first place?

In nematodes, the reduction in the level of X-linked gene expression in 2X animals requires four autosomal genes, *dpy-21*, *dpy-26*, *dpy-27*, and *dpy-28* (26). A fifth gene, *dpy-30*, has also been identified, but its properties have not been published (28). Although the molecular analysis of these genes is still in progress, one possibility is that these genes also encode chromosomal proteins that are either expressed only in 2X animals or localized to the X chromosome only in 2X animals. A peculiarity of these genes is that four of them, the exception being *dpy-21*, have very strong maternal effects on both gene expression and viability. This implies that these gene products are present, but inactive, during the time in which the X:A ratio is being read and are then activated in 2X animals for dosage compensation. An additional unexplained feature is that the maternal absence of *dpy-21*, *dpy-26*, *dpy-27*, or *dpy-28* causes triploids with two X chromosmes to develop as hermaphrodites rather than as males (25, 71). This sex determination effect on triploids may arise from the overexpression of some (unidentified) X-linked numerator (25, 71) or from a feedback from dosage compensation to sex determination (28).

The mutants of *Drosophila* and *C. elegans* that fail to compensate appear to offer the best opportunity for insight into the process of dosage compensation, which in turn seems likely to reveal widespread aspects of gene regulation that are otherwise difficult to detect.

Mammals

How similar are mammalian sex determination and dosage compensation to what has been found in flies and worms? It is clear that a mammalian counterpart of the X:A ratio exists and plays a role in dosage compensation (72). In contrast to flies and worms, mammalian dosage compensation results in inactivation of all but one X chromosome in diploid individuals, with the other X chromosomes inactive and visible cytologically as Barr bodies (3). On the other hand, polyploid cells can have more than one active X chromosome, indicating that the signal to count the chromosomes for inactivation is dependent on the X:A ratio (73). The active and inactive X chromosomes differ in many properties, including methylation patterns and timing of replication. The inactive X chromosome is also lacking in acetylated histone H4 (74). Any of these properties could be the cause of dosage compensation or may simply be consequences of the overall dosage compensation mechanism.

Inactivation of the X chromosome depends on a particular region of the X chromosome termed the inactivation center or Xic (75). The Xic region of the inactive X produces a large transcript Xist (76), an RNA with very limited protein coding capacity (76, 77). Xist RNA is stable and is associated with Barr bodies in the nucleus, which has led to the hypothesis that the functional Xist product is its RNA rather than a protein (78). The role of Xist RNA in X-chromosome inactivation is not clear, although it could initiate X-chromosome inactivation, interact with a protein that controls inactivation, or merely be a reflection of unique chromosomal properties of the Xic region on the inactive X chromosome.

It is not so obvious, however, that the X:A ratio plays a critical role in mammalian sex determination, but there is some evidence that it may. Mammalian embryos have bipotential gonadal primordia. Germline sex differentiation depends primarily on a dominant Y-linked gene *Sry* that triggers formation of the testis (1, 2, 79). Without a Y chromosome, female development ensues. The *Sry* gene encodes a protein with high-mobility group (HMG) domains and binds to DNA, suggesting that transcriptional regulation is important in testis differentiation (1, 79). However, whether *Sry* works alone or is at the head of a hierarchy is not yet known, nor are any targets of *Sry* regulation.

Once *Sry* induces its differentiation, the

Sertoli cells, specialized somatic cells of the testis, synthesize male-promoting hormones that provide an inductive signal to the surrounding somatic cells. Because the signal for mammalian somatic sex determination is an inductive one, concordance between germline sex and morphological sex is usually achieved. However, examination of individuals with gonadal dysgenesis has raised the possibility that sex determination may rely on X-linked genes in addition to *Sry* (*80*). For example, chromosomal duplications that include region Xp21–Xp22.3 of the X chromosome cause XY humans to develop as females, despite having an intact *Sry* gene and an apparently normal Y chromosome. Duplication of this chromosomal region does not inhibit male development, as XXY humans are males. Thus, mammalian germline sex determination is affected by the dosage of critical X-linked genes. This region may contain a target of *Sry* regulation, or it may be involved in sex chromosome counting.

Although flies, worms, and mammals make straightforward decisions between two alternative states, namely female and male, the overall mechanisms of sex determination do not appear to be conserved. In fact, the mechanisms used run the gamut of types of regulation used in cells. Hence, the study of sex determination has yielded insight on two levels: (i) on the overall mechanisms of sex determination itself within these species, and (ii) on particular aspects of gene regulation. The lessons for which sex determination have been most instructive include the importance and role of heterodimeric transcription factors and the mechanisms of alternative splicing. More recent analysis will undoubtedly yield important new information on the regulation of gene expression by 3′ untranslated regions and inductive signaling pathways. Because aspects of sex determination and dosage compensation remain unexplained in all of these organisms, the future promises more lessons to come.

REFERENCES AND NOTES

1. A. H. Sinclair *et al.*, *Nature* **346**, 240 (1990).
2. P. Berta *et al.*, *ibid.* **348**, 448 (1990); J. Gubbay *et al.*, *ibid.* **346**, 245 (1990); J. Gubbay, P. Koopman, J. Collignon, P. Burgoyne, R. Lovell-Badge, *Development* **109**, 647 (1990); P. N. Goodfellow and R. Lovell-Badge, *Annu. Rev. Genet.* **27**, 71 (1993).
3. Reviewed in: S. G. Grant and V. M. Chapman, *Annu. Rev. Genet.* **22**, 199 (1988).
4. C. B. Bridges, *Science* **54**, 252 (1921); *Am. Nat.* **59**, 127 (1925); T. Dobzhansky and J. Schultz, *J. Genet.* **28**, 233 (1934).
5. Reviewed in: B. S. Baker, *Nature* **340**, 521 (1989); J. Hodgkin, *ibid.* **344**, 721 (1990); M. Steinmann-Zwicky, H. Amrein, R. Nothiger, *Adv. Genet.* **27**, 189 (1990); S. M. Parkhurst and D. Ish-Horowicz, *Curr. Biol.* **2**, 629 (1992); T. W. Cline, *Trends Genet.* **9**, 385 (1993).
6. L. R. Bell, E. M. Maine, P. Schedl, T. W. Cline, *Cell* **55**, 1037 (1988).
7. D. Bopp, L. R. Bell, T. W. Cline, P. Schedl, *Genes Dev.* **5**, 403 (1991).
8. L. N. Keyes, T. W. Cline, P. Schedl, *Cell* **68**, 933 (1992).
9. L. R. Bell, J. I. Horabin, P. Schedl, T. W. Cline, *ibid.* **65**, 229 (1991); M. E. Samuels, P. Schedl, T. W. Cline, *Mol. Cell. Biol.* **11**, 3584 (1991).
10. J. W. Erickson and T. W. Cline, *Genes Dev.* **7**, 1688 (1993).
11. T. W. Cline, *Genetics* **113**, 641 (1986).
12. ———, *ibid.* **119**, 829 (1988).
13. M. Torres and L. Sánchez, *EMBO J.* **8**, 3079 (1989); J. W. Erickson and T. W. Cline, *Science* **251**, 1071 (1991); M. Torres and L. Sánchez, *Development* **113**, 715 (1991).
14. S. M. Parkhurst, D. Bopp, D. Ish-Horowicz, *Cell* **63**, 1179 (1990).
15. S. M. Parkhurst, H. D. Lipshitz, D. Ish-Horowicz, *Development* **117**, 737 (1993).
16. J. B. Duffy and J. P. Gergen, *Genes Dev.* **5**, 2176 (1991); M. Torres and L. Sánchez, *Genet. Res.* **59**, 189 (1992).
17. H. Kagoshima *et al.*, *Trends Genet.* **9**, 338 (1993).
18. S. Younger-Shepherd, H. Vaessin, E. Bier, L. Y. Jan, Y. N. Jan, *Cell* **70**, 911 (1992); E. Bier, H. Vaessin, S. Younger-Shepherd, L. Y. Jan, Y. N. Jan, *Genes Dev.* **6**, 2137 (1992).
19. C. Cronmiller and T. W. Cline, *Dev. Genet.* **7**, 205 (1986); C. Cronmiller, P. Schedl, T. W. Cline, *Genes Dev.* **2**, 1666 (1988); M. Caudy *et al.*, *Cell* **55**, 1061 (1988).
20. C. V. Cabrera and M. C. Alonso, *EMBO J.* **10**, 2965 (1991); M. Van Doren, H. M. Ellis, J. W. Posakony, *Development* **113**, 245 (1991).
21. H. M. Ellis, D. R. Spann, J. W. Posakony, *Cell* **61**, 27 (1990); J. Garrell and J. Modolell, *ibid.*, p. 39.
22. R. Benezra, R. L. Davis, D. Lockson, D. L. Turner, H. Weintraub, *ibid.*, p. 49.
23. E. L. Bengal *et al.*, *ibid.* **68**, 507 (1992); W. Gu *et al.*, *ibid.* **72**, 309 (1993).
24. M. D. Perry *et al.*, *Genes Dev.* **7**, 216 (1993).
25. P. M. Meneely, *Genetics*, in press.
26. Reviewed in: A. M. Villeneuve and B. J. Meyer, *Adv. Genet.* **27**, 117 (1990).
27. M. L. Nonet and B. J. Meyer, *Nature* **351**, 65 (1991).
28. L. DeLong, J. D. Plenefisch, R. D. Klein, B. J. Meyer, *Genetics* **133**, 875 (1993); R. D. Klein and B. J. Meyer, *Cell* **72**, 349 (1993).
29. M. McKeown and S. J. Madigan, *Curr. Biol.* **4**, 948 (1992).
30. Reviewed in: J. M. Belote, *Semin. Dev. Biol.* **3**, 319 (1992); K. C. Burtis and M. F. Wolfner, *ibid.*, p. 331.
31. C. Trent *et al.*, *Mech. Dev.* **34**, 43 (1991).
32. P. E. Kuwabara, P. G. Okkema, J. Kimble, *Mol. Biol. Cell* **3**, 461 (1992); P. G. Okkema and J. Kimble, *EMBO J.* **10**, 171 (1991).
33. C. P. Hunter and W. B. Wood, *Nature* **355**, 551 (1992).
34. A. M. Spence, A. Coulson, J. Hodgkin, *Cell* **60**, 981 (1990); J. Ahringer, T. A. Rosenquist, D. N. Lawson, J. Kimble, *EMBO J.* **11**, 2303 (1992).
35. J. Hodgkin, *Genetics* **133**, 543 (1993).
36. D. Zarkower and J. Hodgkin, *Cell* **70**, 237 (1992).
37. ———, *Nucleic Acids Res.* **21**, 3691 (1993).
38. T. Schüpbach, *Genetics* **109**, 529 (1985).
39. M. Steinmann-Zwicky, H. Schmid, R. Nöthiger, *Cell* **57**, 157 (1989).
40. R. Nöthiger, M. Jonglez, M. Leuthold, P. Meier-Gerschwiler, T. Weber, *Development* **107**, 505 (1989).
41. For reviews see: D. Pauli and A. P. Mahowald, *Trends Genet.* **6**, 259 (1990); M. Steinmann-Zwicky, *BioEssays* **14**, 513 (1992); *Semin. Dev. Biol.* **3**, 341 (1992).
42. M. Steinmann-Zwicky, *Development* **120**, 707 (1994).
43. T. Schüpbach, *Dev. Biol.* **89**, 117 (1982); J. L. Marsh and E. Wieschaus, *Nature* **272**, 249 (1978).
44. B. Oliver, Y.-J. Kim, B. S. Baker, *Development* **119**, 897 (1993).
45. B. Granadino, P. Santamaria, L. Sánchez, *ibid.* **118**, 813 (1993).
46. H. K. Salz, T. W. Cline, P. Schedl, *Genetics* **117**, 221 (1987).
47. D. Bopp, J. I. Horabin, R. A. Lersch, T. W. Cline, P. Schedl, *Development* **118**, 797 (1993).
48. M. Steinmann-Zwicky, *ibid.* **117**, 763 (1993).
49. W. R. Steinhauer, R. C. Walsh, L. J. Kalfayan, *Mol. Cell. Biol.* **9**, 5726 (1989); W. R. Steinhauer and L. J. Kalfayan, *Genes Dev.* **6**, 233 (1992); D. Pauli, B. Oliver, A. P. Mahowald, *Development* **119**, 123 (1993).
50. M. Steinmann-Zwicky, *EMBO J.* **7**, 3889 (1988); H. K. Salz, *Genetics* **130**, 547 (1992).
51. B. Granadino, S. Campuzano, L. Sánchez, *EMBO J.* **9**, 2597 (1990); B. Granadino, A. San Juán, P. Santamaria, L. Sánchez, *Genetics* **130**, 597 (1992).
52. D. M. McKearin and A. C. Spradling, *Genes Dev.* **4**, 2242 (1990).
53. T. Preat *et al.*, *Nature* **347**, 87 (1990).
54. B. Oliver, N. Perrimon, A. P. Mahowald, *Genes Dev.* **1**, 913 (1987); B. Oliver, D. Pauli, A. P. Mahowald, *Genetics* **125**, 535 (1990).
55. M. Mével-Ninio, R. Terracol, F. C. Kafatos, *EMBO J.* **10**, 2259 (1991).
56. Reviewed in: J. Kimble and S. Ward, in *The Nematode* Caenorhabditis elegans, W. B. Wood, Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989), pp. 191–213.
57. Reviewed in: J. Hodgkin, *Trends Genet.* **1**, 85 (1985).
58. J. Ahringer and J. Kimble, *Nature* **349**, 346 (1991).
59. E. B. Goodwin, P. G. Okkema, T. C. Evans, J. Kimble, *Cell* **75**, 329 (1993).
60. R. Ellis and J. Kimble, personal communication.
61. M. K. Barton and J. Kimble, *Genetics* **125**, 29 (1990).
62. T. Schedl and J. Kimble, *ibid.* **119**, 43 (1988).
63. P. L. Graham and J. Kimble, *ibid.* **133**, 919 (1993).
64. Reviewed in: J. C. Lucchesi and T. Skripsky, *Chromosoma (Berl.)* **82**, 217 (1981); E. Jaffe and C. Laird, *Trends Genet.* **2**, 316 (1986).
65. Reviewed in: J. C. Lucchesi and J. E. Manning, *Adv. Genet.* **24**, 371 (1987).
66. M. I. Kuroda, M. J. Kernan, R. Kreber, B. Ganetzky, B. S. Baker, *Cell* **66**, 935 (1991); M. J. Palmer *et al.*, *Genetics* **134**, 545 (1993).
67. M. Gorman, M. I. Kuroda, B. S. Baker, *Cell* **72**, 39 (1993).
68. J. R. Bone *et al.*, *Genes Dev.* **8**, 96 (1994).
69. S. Henikoff and P. M. Meneely, *Cell* **72**, 1 (1993).
70. B. M. Turner, A. J. Birley, J. Lavender, *ibid.* **69**, 375 (1992).
71. J. D. Plenefisch, L. DeLong, B. J. Meyer, *Genetics* **121**, 57 (1989).
72. E. M. Eicher and L. L. Washburn, *Annu. Rev. Genet.* **20**, 327 (1986); P. N. Goodfellow and S. M. Darling, *Development* **102**, 251 (1988).
73. B. R. Migeon, J. A. Sprenkle, T. T. Do, *Cell* **18**, 637 (1979).
74. P. Jeppesen and B. M. Turner, *ibid.* **74**, 281 (1993).
75. Reviewed in: A. Ballabio and H. F. Willard, *Curr. Opin. Genet. Dev.* **2**, 439 (1992).
76. C. J. Brown *et al.*, *Nature* **349**, 82 (1991).
77. N. Brockdorff *et al.*, *Cell* **71**, 515 (1992).
78. C. J. Brown *et al.*, *ibid.*, p. 527; G. F. Kay *et al.*, *ibid.* **72**, 171 (1993).
79. P. Koopman, J. Gubbay, N. Vivian, P. N. Goodfellow, R. Lovell-Badge, *Nature* **351**, 117 (1991).
80. T. Ogata *et al.*, *J. Med. Genet.* **29**, 226 (1992); P. Fechner *et al.*, *J. Clin. Endocrinol. Metab.* **76**, 1248 (1993).

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