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REVIEW ARTICLE

Structure, function and evolution of sex-determining systems in Dipteran insects

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SUMMARY

Nature has evolved an astonishing variety of genetic and epigenetic sex-determining systems which all achieve the same result, the generation of two sexes. Genetic and molecular analyses, mainly performed during the last 20 years, have gradually revealed the mechanisms that govern sexual differentiation in a few model organisms. In this review, we will introduce the sex-determining system of *Drosophila* and compare the fruitfly to the housefly *Musca domestica* and other Dipteran insects. Despite the ostensible variety, all these insects use the same basic strategy: a primary genetic signal that is different in males and females, a key gene that responds to the primary signal, and a double-switch gene that eventually selects between two alternative sexual programmes. These parallels, however, do not extend to the molecular level. Except for the double-switch gene *doublesex* at the end of the cascade, no functional homologies were found between more distantly related insects. In particular, *Sex-lethal*, the key gene that controls sexual differentiation in *Drosophila*, does not have a sex-determining function in any other genus studied so far. These results show that sex-determining cascades, in comparison to other regulatory pathways, evolve much more rapidly.

Key words: Sex determination, Evolution, Diptera

INTRODUCTION

Sexual differentiation is a fundamental characteristic of life, affecting almost every aspect of an organism. The sexes consistently differ in physiology and behaviour, and often, but not always, also in morphology and colour. The mystery of two sexes, how they arise and how the sexual dimorphism is produced, has puzzled scientists and laymen alike for centuries. While it is still a matter of debate why sex came into existence, we now begin to understand how sex-determining mechanisms work and how they evolve.

Sexual differentiation has become a paradigm for the genetic control of a developmental pathway. The fascinating insights that we have today are mainly derived from four groups of organisms in which the analysis has proceeded down to the molecular level. These are the fruitfly *Drosophila melanogaster*, the nematode *Caenorhabditis elegans*, the mammals man and mouse, and the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. A comparison of these four systems reveals remarkable parallels and differences (Nöthiger and Steinmann-Zwicky, 1992).

This review is written for the "sex novice" who wants to see the principles of sex determination, rather than the details. For these, the reader is referred to more specialized reviews, notably the one by Cline and Meyer (1996) or the issue "Evolution of sex" (Science Vol. **281** No. 5385, 1998). In this article, we will limit ourselves to Dipteran insects. Even within this order, however, we encounter a perplexing variety of apparently different sex-determining systems (Nöthiger and Steinmann-Zwicky, 1985; Marín and Baker, 1998). Nevertheless, a common principle emerges behind the phenomena: a primary signal, which can be genetic or environmental and which is different in males and females, leads to differential expression of a key gene. The state of activity of this key gene is transmitted through a short cascade of subordinate regulatory genes to the sex differentiation genes which finally transform the molecular signal into the concrete sexual phenotype (Fig. 1).

Does such a common strategy imply that the genes and molecular mechanisms have been conserved in evolution? The disappointing answer, provided by molecular and genetic analyses, is no. Although *Drosophila* and *Caenorhabditis* both use the X:A ratio as the primary signal, the molecular nature of the regulatory genes, and the mechanisms by which these genes control sexual development, are very different (Hodgkin, 1990; Cline and Meyer, 1996). But differences in the regulatory systems exist even between much more closely related species, as we will see in this article.

In contrast, apparently different modes of sex determination can conceal a common genetic and molecular basis. This is the case in *Musca domestica* as a consequence of simple mutations in a few control genes (Table 1). Mutations in any of these

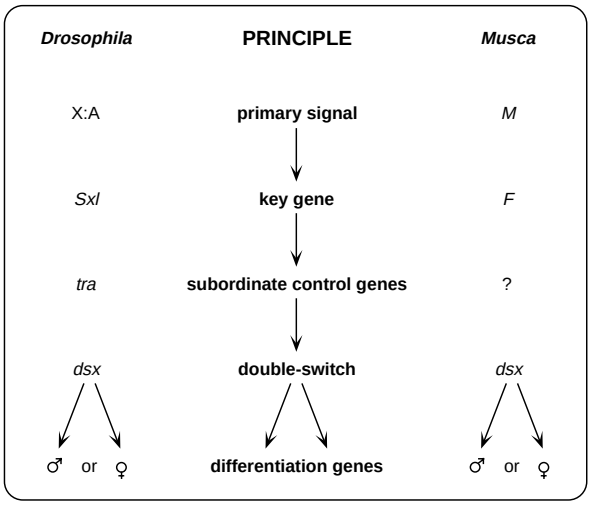

Fig. 1. One strategy, but different mechanisms. The principle and the cases of *Drosophila* and *Musca* are shown. Both species use a primary signal, a key gene and a double-switch. This similarity, however, is not matched by corresponding parallels at the molecular level. The only conserved gene is the final switch gene *dsx*. A homologue of *tra* is not known, and it is also not known whether *F* is directly regulated by *M*. Although a homologue of *Sxl* is present in *Musca*, its expression in both sexes is incompatible with a switch role in sex determination. The recruitment of *Sxl* as the key gene for somatic sex determination in *Drosophila* must be a relatively recent event; the function of *Sxl* in *Musca* is unknown.

genes give the superficial impression that we are dealing with very different mechanisms of sex determination. In this case, however, where five different modes of sex determination coexist within one species, the genes and developmental mechanisms must be the same in all five variants.

Once the genetic elements that control sexual development are known, it is in fact easy to generate new and very different modes of sex determination. An example is provided by a temperature-sensitive allele of *tra2* in *Drosophila*. When *XX; tra2^{ts}/tra2^{ts}* animals are raised at the permissive temperature of 16°C, they develop as fertile females; when raised at 29°C, they become (sterile) males (Belote and Baker, 1982). Thus, the system of *Drosophila*, a textbook case for genetic sex determination, is transformed into one with environmental sex determination by a point mutation in a single control gene.

We will now present the beautifully worked out case of *Drosophila* and compare it to *Musca domestica* and other Diptera. The aim is to familiarize the reader with the *Drosophila* system, and then to ask how valid the fruitfly is as a general model for insects.

DROSOPHILA MELANOGASTER

Sexual differentiation in *Drosophila melanogaster* comprises sex-specific morphology, physiology and behaviour, dosage compensation and sex-specific development of the germ cells (Fig. 2). All these traits ultimately depend on a chromosomal signal that is different in males (XY) and females (XX) and that results in repression or activation of the key gene *Sex-lethal* (*Sxl*). Downstream of *Sxl*, the short cascade of regulatory genes branches into three pathways controlling sexual differentiation of the soma, dosage compensation, which equalizes the amount of X-chromosomal transcripts in males and females, and development of the germline (Fig. 2A). An active *Sxl* gene implements the female programme for all three pathways, whereas an inactive *Sxl* gene dictates male development (the situation in the germline is somewhat more complex, see later).

Sex determination in the soma

The primary signal

The primary genetic signal for sex determination in somatic cells is the ratio of X chromosomes to sets of autosomes (X:A). A ratio of 1.0 (2X:2A) leads to female, a ratio of 0.5 (1X:2A) to male development. The Y chromosome has no sex-determining function, but is necessary for male fertility. The sole target of this complex X:A signal is the key gene *Sxl*, which is activated in females, but not in males (Bopp et al., 1991). This decisive event occurs early in embryogenesis just prior to blastoderm stage (Fig. 2C). At this time, the X:A ratio is cell-autonomously assessed in every somatic cell. The cell-autonomy of sex determination is demonstrated by sexual mosaics. Gynandromorphs, which consist of XX and X0 cells,

Table 1. *Musca domestica*: variations on a theme

	Sex-determining system	Genotype of Males	Genotype of Females
standard strains **autosomal strains**	male heterogamety	*XY^M* *XX; M/+*	*XX* *XX; +/+*
female epistasis ***man* strains**	female heterogamety	*XX; M/M; +/+* *XX; F^{man}/F^{man}*	*XX; M/M; F^D/+* *XX; F^{man}/+*
***Ag* strains**	maternal effect	*XX; Ag/+* or *XX; +/+*	*XX; Ag /+* are arrhenogenic, produce only sons or *XX; +/+* are thelygenic, produce only daughters

Sex determination in *Musca*: standard strain and four variations that are all based on one common mechanism. *M* is the primary signal and *F* is the key gene. An active *F* selects the female pathway. *M* prevents activity of *F*, which leads to male development. *M* has properties of a mobile element and can be located on any chromosome. For review, see Dübendorfer (2000).

F^D is a constitutive mutation that renders *F* active, thus dictating female development, despite presence of *M*.

F^{man} is a loss-of-function mutation so that *F* is inactive, thus dictating male development, even in absence of *M*.

Ag prevents activation of zygotic *F* by a maternal effect.

develop female and male structures side by side. Since *Sxl* controls somatic sex determination, dosage compensation and oogenesis, mutations in this gene not only lead to sexual transformation, but also to death caused by overexpression or underexpression of X-chromosomal genes, and to female sterility due to disruption of oogenesis.

Early in this century, Bridges proposed that sex in *Drosophila* is determined by a balance between female-determining genes on the X chromosome and male-determining genes on the autosomes (Bridges, 1925). For 70 years, this "balance" remained mysterious. Only in the last few years, the components of the X:A signal and their functions were identified (reviewed by Cline, 1993). The genes forming the signal fall into three classes: numerators, denominators and maternal elements all of which encode transcription factors.

(1) The **numerator** genes are located on the X chromosome and are thus present in two copies in females (XX), but only in one copy in males (XY). A gene qualifies as a numerator when it fulfils the following operational criteria: duplications of the numerator elements in XY zygotes lead to activation of *Sxl* causing male-specific lethality; this effect can be compensated by loss-of-function mutations in *Sxl*. Deletions of numerator genes in XX zygotes prevent activation of *Sxl*, which leads to female-specific lethality; such females can be rescued by a constitutive gain-of-function mutation of *Sxl*. So far, four different numerator genes have been identified: three *sisterless* genes (*sisA*, *sisB*, *sisC*) and *runt* (*run*) (Cline, 1988; Duffy and Gergen, 1991; Erickson and Cline, 1993; Deshpande et al., 1995). The different numerators act in a semiadditive manner inasmuch as increased dose of one element can partially compensate for decreased activity of another element.

(2) The single known **denominator** gene *deadpan* (*dpn*) is a transcription factor that negatively regulates *Sxl*. As a denominator, *dpn* is located on an autosome. Increased dose of *dpn*, combined with an otherwise viable reduction of the numerators, leads to female-specific lethality; conversely, mutations in *dpn* cause a reduced viability of males (Barbash and Cline, 1995). The products of the numerators *sisB* and *run* and of the denominator *dpn* have been shown to bind to specific sites in the promoter of *Sxl*, thereby activating or repressing the gene, respectively (Hoshijima et al., 1995; Kramer et al., 1999).

(3) At least four **maternal genes** help to interpret the X:A signal. The products of *daughterless* (*da*) and *hermaphrodite* (*her*) are positive regulators of *Sxl* (Cronmiller et al., 1988; Pultz and Baker, 1995), while those of *extramacrochaetae* (*emc*) and *groucho* (*gro*) are negative regulators (Younger-Shepherd et al., 1992; Paroush et al., 1994). Maternal DA and zygotic SISB proteins form heterodimers (Cabrera and Alonso, 1991) that function as transcriptional activators of *Sxl* (Parkhurst et al., 1990). XX embryos from *da* mutant mothers lacking maternal DA protein fail to activate *Sxl* and die. The GRO protein is thought to act as a corepressor by binding to DPN. EMC probably sequesters the positive regulators DA and/or SISB by forming inactive heterodimers (Van Doren et al., 1991).

Neither the denominator(s) nor the maternal components have discriminative power in *Drosophila*. They are present in equal doses in both sexes and serve to transduce the signal produced by the numerators. The numerators can play their

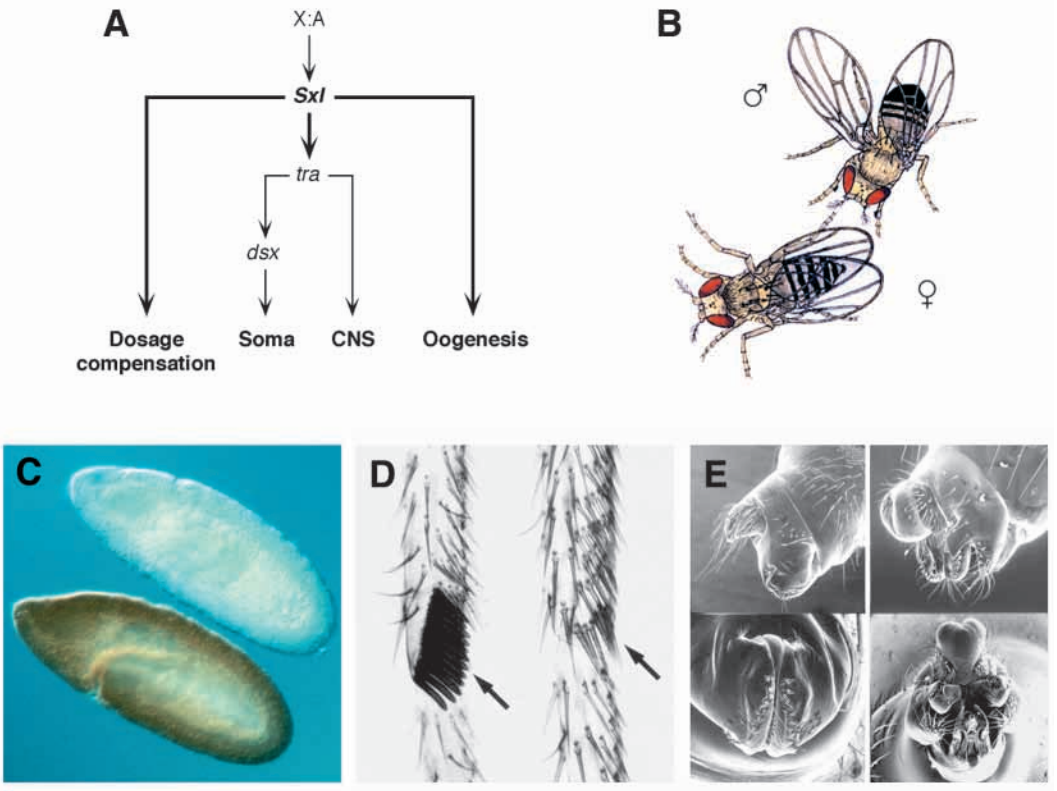

Fig. 2. Sexual dimorphism in *Drosophila*. (A) Schematic representation of the role of *Sxl* in dosage compensation, somatic sexual development and oogenesis. The sex of the central nervous system (CNS) and of the rest of the soma is controlled by *tra*, but the pathway branches downstream of this gene. *Sxl* achieves its effects via a small number of subordinate control genes. For more details see Fig. 6. Sexual dimorphism is manifested in behavioural, physiological and morphological differences between the sexes. (B) A male, recognizable by its darkly pigmented 5th and 6th tergites, courts a female by vibrating with one of its wings, generating the "love song". (C) Expression of SXL protein in female (brown), but not in male embryos. (D) The basitarsus of the foreleg of males (left) carries a "sex comb" (arrow) of some 12 heavy blunt bristles, rotated by 90° relative to the rest of bristle rows. The sex comb corresponds to two unrotated normal bristle rows (arrow) in females (right). (E) Analia and genitalia of a female (left) and a male (right), viewed from the side (upper row) and from posterior-ventral (lower row).

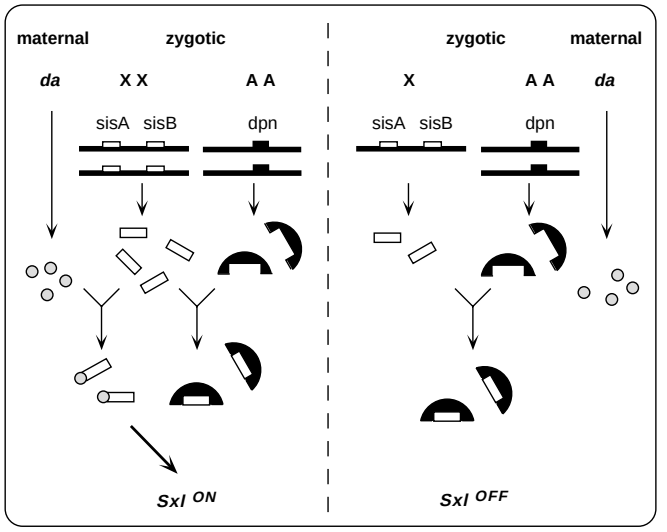

Fig. 3. Simplified titration model to illustrate how the X:A ratio regulates transcription of *Sxl* in *Drosophila*. The discriminating factors are the X-chromosomal numerators: if the ratio is 0.5, all numerator molecules (only SISA and SISB are drawn) are bound by the negatively acting denominator (DPN); if the ratio is 1.0, some numerators remain free and can form a heteromeric complex with maternal DA. This complex activates transcription of *Sxl* (Parkhurst and Ish-Horowicz, 1992). The reality is more complex. In addition to the titration effect, the different positive and negative transcription factors compete directly for multiple binding sites on *Sxl* by cooperative binding (Estes et al., 1995).

decisive role because dosage compensation is not yet established at the time that the signal is assessed so that females have twice as much numerator products as males.

How can an X:A ratio of 0.5 or 1.0, i.e. a quantitative difference of 1 versus 2, be transduced into an all-or-none response of *Sxl*? A simple titration model in which positive and negative regulators compete for heterodimerization may reveal the principle (Fig. 3).

Initiation and maintenance of *Sxl*

Shortly before blastoderm formation, an establishment promoter of *Sxl*, Sxl_{Pe}, is activated in XX embryos by the X:A signal. This leads to the production of early SXL protein (Keyes et al., 1992; Estes et al., 1995). Slightly later, transcription from Sxl_{Pe} ceases and a maintenance promoter, Sxl_{Pm}, becomes active in both sexes. From now on, sex-specific expression of *Sxl* is achieved by alternative splicing (Fig. 4A). Male transcripts retain the third exon which contains stop codons so that translation terminates prematurely (Bell et al., 1988, 1991; Horabin and Schedl, 1993). In females, this exon is spliced out, which leads to a complete open reading frame. The driving force for female-specific splicing is SXL protein itself which thus initiates an autoregulatory loop. Since males produce no early SXL protein, exon 3 cannot be spliced out and autoregulation is not established. If, however, a boost of SXL protein is provided by experimentally activating a transgene of female-specifically spliced cDNA in XY males, the autoregulatory loop can be set in motion. Such males now continuously produce SXL and eventually die from underexpression of X-chromosomal genes (Bell et al., 1991).

In addition to *Sxl* itself, three other genes are known to be required for autoregulation of *Sxl*. The gene *sans fille* (*snf*, also called *splicing necessary factor*) encodes a general splicing factor homologous to the mammalian U1A and U2B″ proteins (Albrecht and Salz, 1993; Flickinger and Salz, 1994). In combination with SXL, SNF blocks the splice sites of exon 3, which is thus treated as an intron (Fig. 4B). The two other genes involved in autoregulation, *female-lethal(2)d* (*fl(2)d*) and *virilizer* (*vir*), encode proteins with unknown functions, and their role in processing the *Sxl* transcripts is not understood (Granadino et al., 1992; Hilfiker et al., 1995). Loss of function of any of these genes, *snf*, *fl(2)d* or *vir*, results in male-specific splicing of *Sxl* transcripts in XX embryos causing their death due to overexpression of X-linked genes. Although XX animals that lack *Sxl* activity die, the sex-transforming effect of mutations in *Sxl*, *fl(2)d* or *vir* can be visualized in genetic mosaics. Cell clones homozygous for *Sxl*, *fl(2)d* or *vir* generated in heterozygous females are viable and differentiate male structures.

The complex regulation of *Sxl* with the promoter swap and switch to autoregulation is an elegant way to solve a fatal problem that the fly would otherwise face during later development. Once the state of activity of *Sxl* is established, dosage compensation equalizes the X-chromosomal products including those of the numerator genes in males and females. This is necessary because most genes involved in the primary signal of sex determination have other functions in later development for which their products have to be present in equal amounts in males and females. If *Sxl* were still sensitive to transcriptional regulation by the numerator products, it would now be activated in XY animals, with deadly consequences.

Transmission of the primary signal and execution of the programme

Consistent with its function in RNA processing, *Sxl* is an RNA-binding protein with two conserved RRM domains (RNA recognition motif). The protein not only maintains its own activity, but also controls the expression of downstream genes at the top of three regulatory cascades (Fig. 2A). Two of the target genes and the mechanisms by which *Sxl* regulates them are known. These are *transformer* (*tra*) for somatic sex determination and *male-specific lethal-2* (*msl-2*) for dosage compensation. For the third pathway, oogenesis, no target gene is known yet. We will first deal with the somatic sexual pathway.

Similar to *Sxl*, *tra* produces a pre-mRNA in both sexes. A productive splice leading to an mRNA with an open reading frame, however, requires SXL which is only present in females (Fig. 4A,B) (Sosnowski et al., 1989). Together with the product of the constitutively active gene *transformer2* (*tra2*) (Amrein et al., 1988), TRA protein promotes female-specific splicing of the bifunctional gene *doublesex* (*dsx*) which again is transcribed in both sexes (Fig. 4A) (Hoshijima et al., 1991). TRA and TRA2 both resemble SR (splicing regulator) proteins, and they promote spliceosome assembly at the suboptimal 3′ splice site upstream of exon 4, which is thus retained in females. In the absence of TRA or TRA2, the transcripts of *dsx* are male-specifically spliced.

The two products of *dsx*, DSXM and DSXF, are transcription factors that control the activity of the final target genes

necessary for sexual differentiation. They share the first three exons, but differ at their C-terminal ends (Burtis and Baker, 1989). The common sequence contains a DNA-binding domain by which the DSX proteins bind to their target genes; the C terminus is responsible for the sex-specific effect on these genes (Fig. 5). DSXM represses genes required for female differentiation and activates the male differentiation genes; and DSXF represses male-specific and activates female-specific genes. Two other genes, *her*, which we already introduced as a component of the X:A signal, and *intersex* (*ix*), are required for the activity of DSXF, but their molecular functions are still unknown (Pultz et al., 1994; Chase and Baker, 1995).

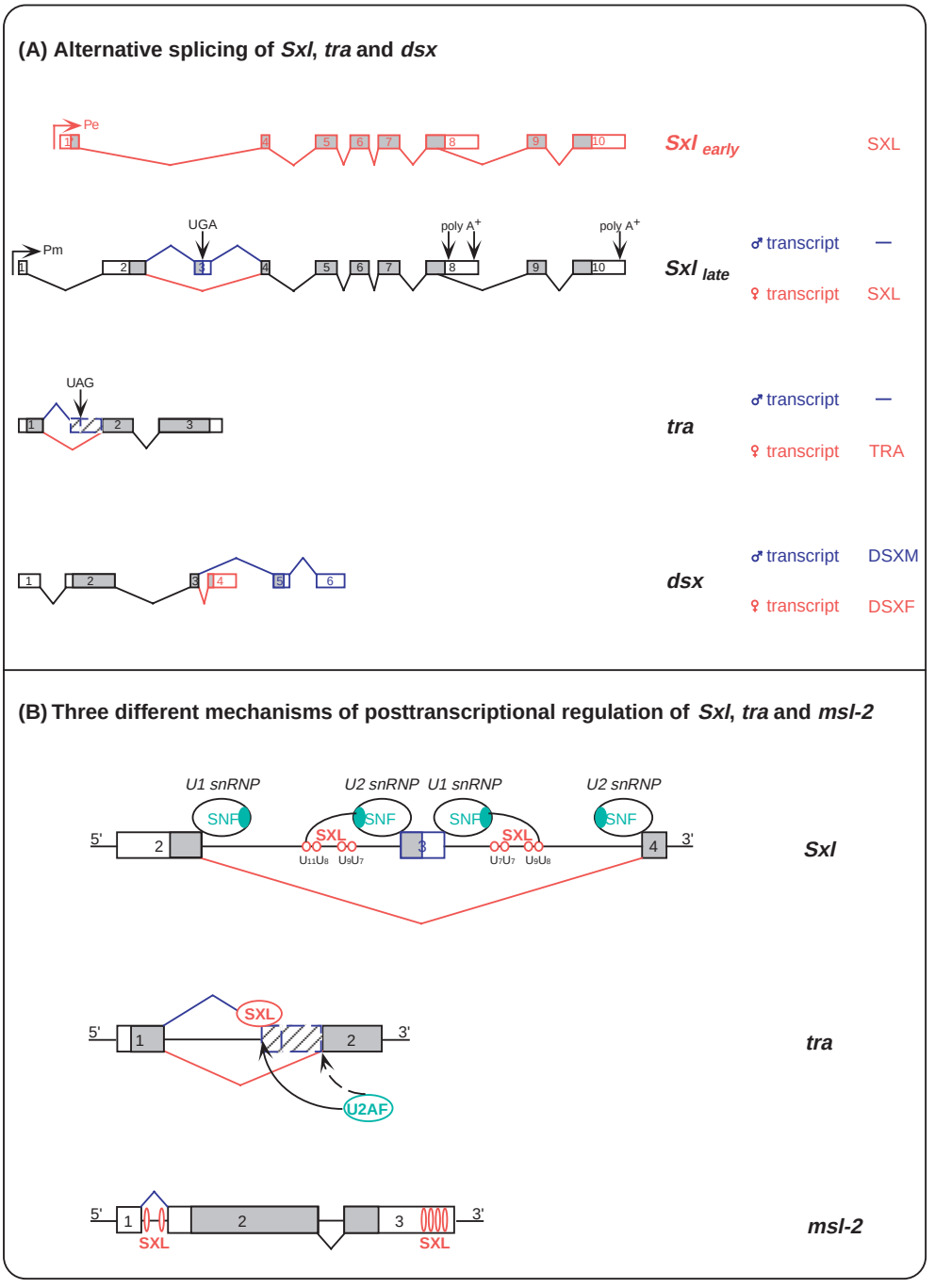

Fig. 4. Transcripts and proteins of some of the sex-determining genes. Female transcripts and proteins are in red, male products are in blue. Open boxes represent the untranslated parts of the exons, horizontal lines in B represent introns. (A) SXL proteins deriving from the early and from the late transcripts are identical except for the first ~25 amino acids. The early products of *Sxl* arise by transcriptional regulation and are present only in females. The late products of *Sxl* as well as those of *tra* and *dsx* arise by post-transcriptional regulation through alternative splicing. The male mRNAs of *Sxl* and *tra* contain an exon with stop codons leading to a truncated and non-functional protein. SXL protein is responsible for female-specific splicing of its own transcripts and of those of *tra*. The male and female mRNAs of *dsx* share the first three exons. The female mRNA ends with exon 4. This exon is skipped in males whose message terminates with exons 5 and 6. (B) *Sxl* controls alternative splicing of itself, *tra* and *msl-2* by three different molecular mechanisms: exon skipping, alternative choice of 3′ splice sites and intron retention, respectively (for a review on alternative splicing see Lopez, 1998). In all three cases, SXL protein prevents a male-specific splice. (1) Autoregulation of *Sxl* (modified from Deshpande et al., 1996). SXL protein binds to the poly(U) tracts in the introns upstream and downstream of the male-specific exon 3. Via protein-protein interaction with SNF, SXL makes contact with the U1 and U2 snRNPs, which are associated with the 5′ and 3′ splice sites of the male-specific exon, respectively. This complex masks the splice sites on both sides of exon 3 so that the 5′ splice site of exon 2 and the 3′ splice site of exon 4 are joined leading to the excision of exon 3. (2) Alternative choice of 3′ splice sites in *tra*. The hatched box represents the sequence that is spliced out in females. SXL competes with the auxiliary splicing factor U2AF for binding at the more upstream polypyrimidine tract. In the presence of SXL, the upstream splice site is blocked, and U2AF promotes splicing at the weaker downstream splice site. (3) Direct translational regulation of *msl-2*. The *msl-2* transcript contains two SXL-binding sites in the 5′ untranslated region (UTR) and four SXL-binding sites in the 3′ UTR. By binding to these sites, SXL directly prevents translation of *msl-2* in females (Bashaw and Baker, 1997; Kelley et al., 1997). Deletion of the SXL-binding sites leads to constitutive expression of *msl-2*. In males, in absence of SXL, the small intron between exons 1 and 2 is spliced out, but its removal is not required for translation of *msl-2*.

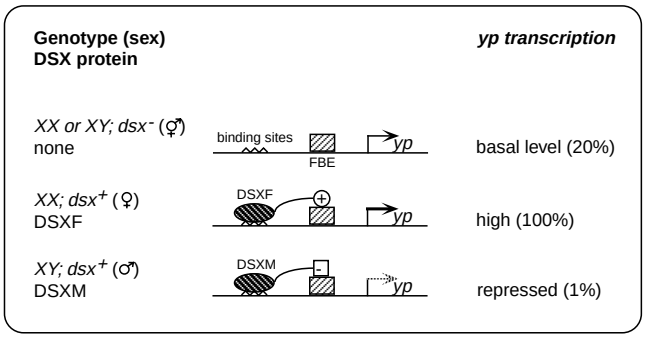

Fig. 5. Regulation of the *yp* genes in the fat body (simplified model from Coschigano and Wensink, 1993). The key to the tissue- and stage-specificity is an activator protein (hatched rectangle) that is only made in the fat body cells of adult flies. In the absence of any DSX protein in mutant *dsx* flies (which develop as intersexes ⚥), the binding of this activator protein to the fat body-specific enhancer (FBE) confers a basal transcriptional activity onto the *yp* genes. DSXF strongly enhances (+) this activity while DSXM represses (−) it. Both DSX proteins bind as dimers to the same two adjacent binding sites (for simplicity, only one is drawn) (An and Wensink, 1995; Cho and Wensink, 1997, 1998); their opposite effects on YP synthesis result from the sex-specific C terminus (circle or rectangle).

Most somatic sexual characters are determined by *dsx*; but there are exceptions. The sex of the CNS, i.e. sexual behaviour and neural induction of a male-specific abdominal muscle, depends on the state of activity of *tra*, not *dsx* (Taylor et al., 1994). The instructions given by *tra* are mediated by the genes *fruitless* (*fru*) and *dissatisfaction* (*dsf*) which again, like *dsx*, encode transcription factors (Ryner et al., 1996; Finley et al., 1997). This suggests that they may also directly control the final, but yet unknown target genes in the developing CNS.

Since most sexual traits depend on *dsx*, this gene must regulate a number of genes necessary for sexual differentiation. So far, however, only one target, the three *yolk protein* (*yp*) genes, is known. The *yp* genes are expressed in a tissue-, stage- and sex-specific manner, namely in the fat body and in the ovarian follicle cells of adult females. Their regulation demonstrates the two modes by which *dsx* controls differentiation genes. In the fat body, a tissue that is common to both sexes, the *yp* genes are under permanent control of *dsx* (Fig. 5). In XX flies mutant for a temperature-sensitive allele of *tra2*, the *yp* genes can be transcriptionally turned on or off whenever the ambient temperature is shifted between the permissive temperature (leading to DSXF) and the restrictive temperature (leading to DSXM) (Belote et al., 1985). In contrast, in the ovarian follicle cells, which are a female-specific tissue, the *yp* genes remain active even when the flies are shifted to the restrictive temperature (Bownes et al., 1990). Once the follicle cells have formed, the *yp* genes are no longer under direct control of the sex-determining genes. Instead, their regulation has been delegated to a tissue-specific factor. It is the formation of the female-specific tissue, in this case the follicle cells, which requires the activity of TRA2 and DSXF.

In contrast to the genes forming the primary signal, which is only transiently required for the transcriptional control of *Sxl*, all other genes are giving permanent instructions that maintain the cells on the initiated pathway. Clonal analysis revealed that XX cells rendered homozygous mutant for *Sxl*, *tra* or *tra2* any time between blastoderm up to the third larval stage form male structures in the adult fly (Baker and Ridge, 1980; Sánchez and Nöthiger, 1982).

The contents of this chapter are summarized in Fig. 6, which shows the genes and their expression and function in sex determination.

Dosage compensation

The evolution of heteromorphic sex chromosomes creates a problem: a twofold difference in the amount of X-chromosomal gene products between males and females would lead to an imbalance of X-chromosomal and autosomal gene products in one sex, and this imbalance could be fatal. Dosage compensation is a regulatory mechanism that equalizes the transcripts of X-chromosomal genes between the sexes.

In *Drosophila*, dosage compensation is achieved by hyperactivating the single X of males such that it produces the same amount of transcripts as the two X chromosomes in females. In *Caenorhabditis elegans* with XX hermaphrodites and X0 males, the two X chromosomes in hermaphrodites are hypoactive compared to the one X chromosome in males (reviewed by Cline and Meyer, 1996). Hyperactivation in *Drosophila* and hypoactivation in *C. elegans* refer to a basal transcriptional activity of the autosomes. Only if the sex chromosomes carry very few genes, a species can do without dosage compensation. Birds and *Musca domestica* are examples.

Mutations in genes controlling dosage compensation cause sex-specific lethality, but no sexual transformation. In *Drosophila*, five genes, collectively called *male-specific lethal* genes (*msl-1*, *msl-2*, *msl-3*, *mle*, *mof*), are required for hypertranscription of X-chromosomal genes in males (reviewed by Lucchesi, 1996). Loss of function of any of them is lethal for males, but has no effect in females. Surprisingly, four of these five genes are expressed in both sexes, although they are only required in males. The only gene that is expressed exclusively in males and has to be inactive in females is *msl-2* (Bashaw and Baker, 1995; Kelley et al., 1995; Zhou et al., 1995). As stated above, *Sxl* not only regulates sex determination, but also dosage compensation. *msl-2* is the primary target gene of *Sxl* in this pathway. It is again post-transcriptionally regulated and produces a protein only in males (Fig. 4B). All five MSL proteins form a heteromeric complex that localizes to hundreds of specific sites along the X chromosome in males. In the absence of any one of the MSL proteins, the complex does not form. Binding of the MSL complex is correlated with a significant increase of histone 4 acetylated at lysine 16 (H4ac16) localized to the same sites on the X chromosome. H4Ac16 is thought to be responsible for the observed changes in chromatin structure and the resulting hypertranscription (Bone et al., 1994).

The role of *Sxl* in dosage compensation is more complex than suggested by the previous paragraph (Bernstein and Cline, 1994). It appears that *Sxl* achieves dosage compensation by two different mechanisms. Early in embryogenesis, it controls the activity of X-chromosomal genes directly. Binding of SXL to several sites along the X chromosomes in females and the finding of 20 X-chromosomal transcripts with optimal SXL-binding sites, similar to the binding sites in *msl-2*, are consistent with such a direct role in dosage compensation (Kelley et al., 1995). Later in development, *Sxl* acts indirectly

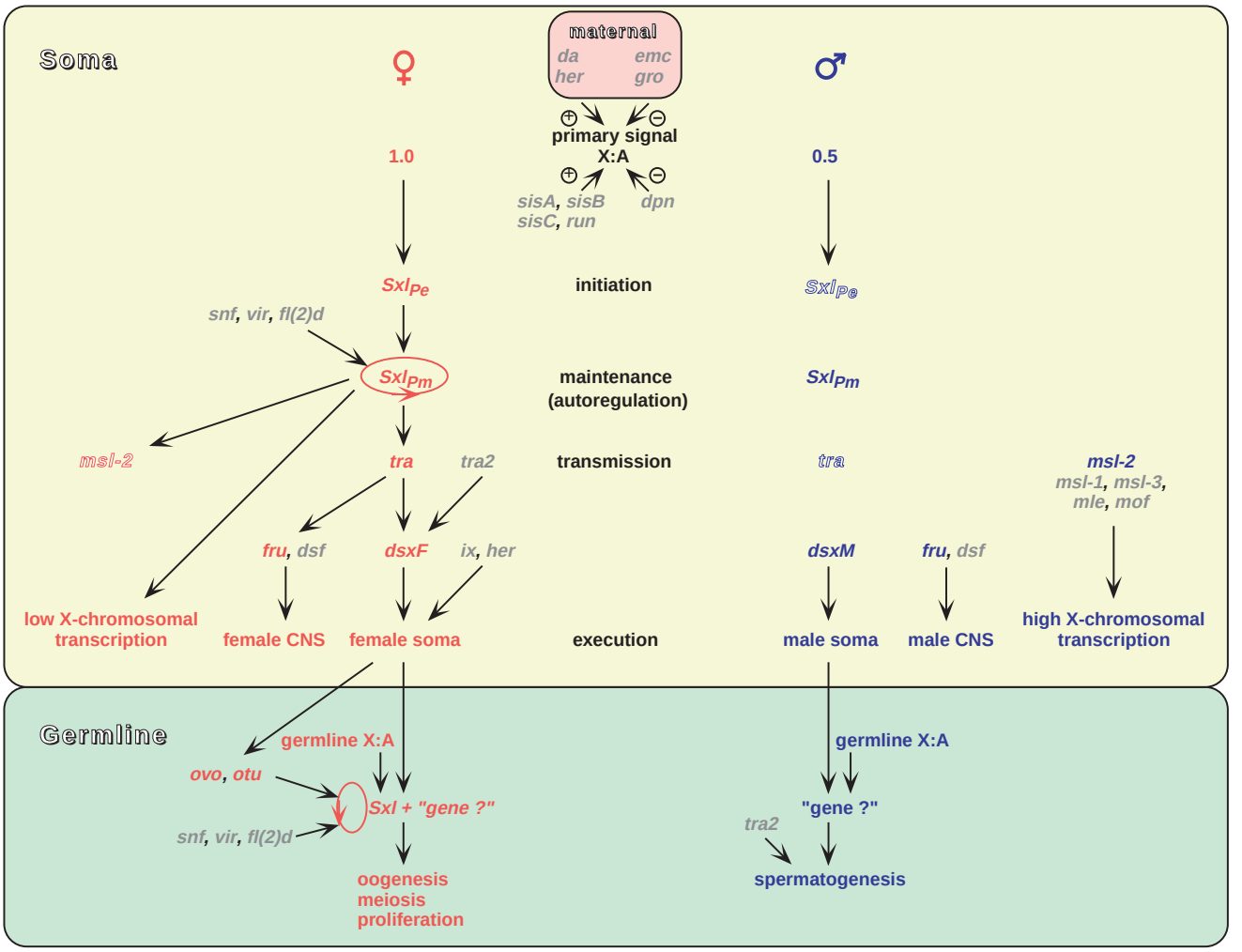

Fig. 6. Genetic network regulating sexual differentiation. Genes specifically expressed in females are in red; the male pathway is outlined in blue. Genes in grey are active in both sexes, but are only listed in the sex in which they have a known sex-specific function. Open gene symbols indicate inactivity. Arrows symbolise the effect (positive or negative) of a gene product on its target. The genes forming the primary signal and *dsx* encode transcription factors; *Sxl*, *tra* and *tra2* encode proteins involved in RNA processing and act at the post-transcriptional level. The lower part of the figure shows the situation in the germline with the non-autonomous somatic and the autonomous germline signals. For description of the genes, their regulation and function see text.

by regulating the expression of *msl*-2. The genes involved in dosage compensation are summarized in Fig. 6.

Sex determination in the germline

The mechanism of sex determination in the germline is still poorly understood (summarized in Fig. 6). In *Drosophila*, a combination of inductive signals from the soma and autonomous information given by the "germline X:A ratio" determines the sexual fate and development of germ cells. This was first shown by heterosexual combinations of somatic cells and germ cells. When XX pole cells, the prospective germ cells, are transplanted into XY hosts, they initiate spermatogenesis, but do not complete it despite a male environment. In contrast, XY pole cells transplanted into XX hosts become spermatogenic, but again undergo abortive development (Steinmann-Zwicky et al., 1989).

Sxl in the germline

The master gene for somatic sex determination, *Sxl*, is also required for oogenesis (Bopp et al., 1993). In the germline, however, it does not function as the key gene of sex determination and its regulation is different from that in the soma. If *Sxl* were the key to sexual development in the germline, XX germ cells lacking *Sxl* function, in analogy to the effect of the gene in the soma, should form sperm, and XY cells with constitutive mutations of *Sxl* should form eggs. This is not the case. XX cells lacking *Sxl* undergo excessive proliferation and develop tumorous cysts; such cells may form spermatocyte-specific crystals, but do not form sperm even if they are transplanted into the appropriate environment of a male host (Schüpbach, 1985; Steinmann-Zwicky et al., 1989). Analogously, XY germ cells with constitutive mutations of *Sxl* are not feminized, but form fertile sperm in male hosts (Steinmann-Zwicky, 1993). Furthermore, first sex-specific differences, e. g. the expression of a male-specific molecular marker, are observed prior to the expression of *Sxl* (Staab et al., 1996). All these results make it unlikely that this gene has a sex-determining function at the top of a cascade.

The initiation of *Sxl* in the germline is different from that in the soma. *Sxl* is activated much later in germ cells than in somatic cells. Neither the known numerator elements *sisA*, *sisB* and *run*, nor the maternal product of *da* are required for the activation of *Sxl* in the germline, indicating that the "germline X:A ratio" uses numerator elements that differ from those operating in somatic cells. The targets downstream of *Sxl* are also different in soma and germline. *tra* and *dsx*, the genes involved in somatic sex determination, are dispensable within germ cells, but they are responsible for the inductive signal given by the somatic cells (Steinmann-Zwicky, 1994; Hinson and Nagoshi, 1999).

Nevertheless, there exists a parallel between soma and germline. The activity of *Sxl* in XX cells seems to be maintained by autoregulation that depends, as in the soma, on the function of *snf*, *fl(2)d* and *vir*. Additionally, two germline-specific genes, *ovo* and *ovarian tumor* (*otu*), are necessary for autoregulation (Granadino et al., 1992; Salz, 1992; Oliver et al., 1993; Pauli et al., 1993; Hager and Cline, 1997; Schütt et al., 1998).

Although there still remain many questions on the regulation and function of *Sxl* in the germline, recent experiments indicate that *Sxl* controls the pattern of mitotic divisions in the ovarian germarium and the transition from mitosis to meiosis. Additionally, the gene is required for meiotic recombination, which is a female-specific trait. Misexpression of *Sxl* in early oogenesis strongly reduces or abolishes crossing over (Bopp et al., 1999).

A key gene for sex determination in germ cells is still not known. Mutations in such a gene are expected to cause sex-specific sterility due to sexual transformation of the germline, but not of the soma. However, no mutation that clearly and completely changes the sexual fate of germ cells is known so far. In fact, such a key gene may not exist. It rather appears that the inductive and autonomous signals control a small number of target genes, each necessary for some aspects of sexual development of germ cells. Therefore, mutations in any of these target genes – *Sxl*, *ovo*, *otu* may belong to this group – lead only to a partial transformation and to the observed expression of both male and female characteristics in one cell. These contradictory instructions may block differentiation, resulting in the frequent phenotype of tumorous cysts.

DROSOPHILA AS *THE* PARADIGM?

As stated in the "Introduction", the sex-determining strategies of various taxonomic groups reveal a common principle: a primary signal controls a key gene at the top of a short regulatory cascade (Fig. 1) (Marín and Baker, 1998). The important question, however, is about the nature of the genes forming the sex-determining cascade. How far have the participating genes and their functions been conserved during evolution?

The search for genes homologous to sex-determining genes of *Drosophila* was both sobering and illuminating. A homologue of *Sxl* was found in *Chrysomya* (Müller-Holtkamp, 1995), *Megaselia* (Sievert et al., 1997), *Musca* (Meise et al., 1998) and *Ceratitis* (Saccone et al., 1998), but it did not qualify as a sex-determining gene in any of these Dipteran insects: SXL proteins of the same size were found in both sexes, an expression pattern that is inconsistent with a discriminatory role in sex determination. Furthermore, despite the high degree of similarity (some 85% of all aminoacids are shared by SXL of *Drosophila* and *Musca*), a transgene carrying a *Musca-Sxl* cDNA had no sex-transforming effect in *Drosophila* although the protein was properly expressed and localized to the nuclei (Meise et al., 1998). Thus, while the structure of *Sxl* is very well conserved, its developmental function is not.

The situation is different for *dsx*, the gene at the end of the cascade. This gene is highly conserved not only in its structure, but also in its expression and possibly also in its function. As in *Drosophila*, the *dsx* homologues of *Megaselia* (Sievert et al., 1997), *Bactrocera* (Shearman and Frommer, 1998), *Musca* (M. Hediger and D. Bopp, personal communication), and *Ceratitis* (G. Saccone and L. Polito, personal communication) express male- and female-specific transcripts, and the exon-intron structure is largely conserved. Although the function of the *dsx* homologue has not yet been tested in these flies, the sex-specific expression pattern is at least suggestive of a sex-determining role.

The described findings are in accord with the model of Wilkins (1995) who proposed that sex-determining cascades are built and are evolving from bottom up, not from top down. Accordingly, the last gene in the cascade is the ancient and conserved gene, and different upstream regulators are recruited by different taxonomic groups during evolution. The structural and functional conservation of *dsx* is impressive. The *mab-3* gene of *Caenorhabditis* is the structural homologue of *dsx*. Similar to DSXM in *Drosophila*, MAB-3 forces male-specific development of peripheral sensory organs and prevents transcription of the *yolk protein* genes in males. More impressively, a *Drosophila* transgene encoding DSXM can partially complement *mab-3* mutations in *Caenorhabditis* males. Different from *Drosophila*, however, *mab-3* encodes no female-specific product corresponding to DSXF (Raymond et al., 1998; Yi and Zarkower, 1999).

The described parallels and differences raise a number of questions that may be experimentally approached:

(1) Why is the last gene, *dsx*, conserved, and why do upstream regulators evolve so rapidly? – It can be safely assumed that the DSX transcription factors control a number of sex differentiation genes whose *cis*-regulatory sequences all have to recognize the DSX protein. Thus, a significant change in the coding region of *dsx* would simultaneously affect the expression of many genes, and this would result in a developmental disaster. This situation puts rigorous constraints on the evolution of the coding region of *dsx*. In contrast, a single upstream regulator, as long as it can interact with the controlling elements of *dsx*, in this case the splice sites, can be relatively freely recruited from among genes encoding splice factors or cofactors of splicing proteins.

(2) What functions do the male- and female-specific products of *dsx* have in the different species, and how is alternative splicing achieved? – We cannot a priori assume that the functions of the gene in other species are the same as in *Drosophila* where it acts as a global regulator of somatic sexual differentiation. It will be easy to test how far a *Musca dsx* cDNA can compensate a *dsx* mutation in *Drosophila*. To find out the function of *dsx* in *Musca* or any other species, however, we have either to knock out the endogenous gene or to ectopically express it. This will become possible once the

new and powerful technique of double-stranded RNA-interference (RNA-i) (Misquitta and Paterson, 1999) is established or a transformation system becomes available which is already the case for *Ceratitis* (Loukeris et al., 1995). Searches for upstream genes regulating the sex-specific processing of the *dsx* transcripts have been unsuccessful so far: no homologue of *tra* was found outside of the genus *Drosophila*. Even within the genus, the coding region of *tra* has surprisingly diverged (O'Neil and Belote, 1992). We expect that the functional homologue of *tra* must encode a protein of the SR type to achieve, together with the constitutive *tra2*, female-specific splicing of *dsx* transcripts. Since *tra2* appears to be widely conserved (Dauwalder et al., 1996; Chandler et al., 1997) and a homologue may be present in *Musca* and even in species much more distantly related, any gene product that could act as a cofactor for TRA2 to form a functional splice complex could serve as a new discriminator if it is expressed only in females. Using the yeast hybrid system, it may be possible to isolate a protein that interacts with TRA2, and thus to find the upstream regulator of *dsx*. In *Musca*, this gene may correspond to *F* which has the predicted genetic properties: it is required for female development and must be inactive for male development (Table 1) (Schmidt et al., 1997).

(3) What is the function of *Sxl* in *Musca* and other Diptera? How was this gene recruited as the key regulator of sexual development in *Drosophila*, and which gene, if any, took over the ancient function of *Sxl* in this species? – *Sxl* is entirely dispensable in *Drosophila melanogaster* males: XY animals with a deficiency of *Sxl* are perfectly viable and fertile. An indication for an ancient function of *Sxl* derives from *D. virilis* which takes an intermediate phylogenetic position between *Musca* and *D. melanogaster*. At blastoderm stage, SXL shows the same female-specific expression pattern in *D. virilis* as in *D. melanogaster* and is probably also controlling sexual development. Later in embryogenesis, however, SXL becomes also expressed in the prospective CNS of *D. virilis* males (Bopp et al., 1996). It is therefore possible that *Sxl* had an original somatic function in neural development. In addition, recent experiments point to an ancestral and probably conserved role of *Sxl* in the germline. In contrast to the soma where a *Musca Sxl* transgene introduced into *Drosophila* has no effect, such a transgene can partially rescue misexpression of *Sxl* in female germ cells (D. Bopp and M. Hirsch, personal communication). The hypothesis of an ancestral function in the germline is supported by the observation that, in *Megaselia*, *Sxl* is expressed only in the gonad (Sievert et al., 1997). Again, RNA-interference should help to reveal the function of *Sxl* in *Musca* and other species where a genetic analysis is not yet possible and where we have only a vague idea what phenotype a mutation would cause.

CONCLUDING REMARKS

Sexual differentiation comprises two problems that are interconnected. Once we have understood how the sex-determining cascade produces its final molecular signal, DSXM or DSXF, we are confronted with the next question: How is this ubiquitous signal transformed into the actual sexual phenotype, i.e. sex combs on the forelegs, synthesis of yolk proteins in the fat body, sexual behaviour in the CNS, etc.? To achieve this regionally different sexual phenotype, DSXM and DSXF must be interpreted in the context of a specific cell type. This cell type is the result of "ordinary" differentiation. At present, we are rather ignorant about this interaction between products of sex-determining genes and cell type. Some light is being shed by analyses of the *yp* genes in *Drosophila* (see text and Fig. 5).

Sex determination and its control is a fascinating problem. We are now beginning to understand the logic and mechanics by which various species achieve sexual differentiation. The analyses reveal a common genetic strategy overlaying an unexpected variety of mechanisms and a lack of conservation of the genes forming the genetic cascades. What is the driving force behind these rapid evolutionary changes? Speculations about this question are found in recent review articles (Hodgkin, 1990; Wilkins, 1995; Marín and Baker, 1998; Werren and Beukeboom, 1998). Evolutionary changes may be facilitated because sex-determining cascades, in contrast to the complex networks regulating segmental identity and tissue-specific differentiation, are essentially linear above *dsx*, and a mutation in any of the global regulators simply changes one sex into the opposite sex without further deleterious effects. This simple view is refuted by *Drosophila*, which appears to have lost its evolutionary flexibility. The reason for this is that *Sxl* controls three pathways, sex determination in the soma, dosage compensation and oogenesis, and that the germline has an autonomous control system and does not simply follow the sexual pathway of the soma. Thus, mutations in any of the genes regulating sexual differentiation lead to sex-specific lethality (dosage compensation) or sterility (incompatibility between the sex of the soma and the sex of the germline). It can thus be predicted that *Drosophila* will remain blocked in its present sex-determining system forever. The sexual development of germ cells is entirely non-autonomous in *Musca* (Hilfiker-Kleiner et al., 1994), *Chrysomya* (Ullerich, 1984) and *Sciara* (Mori and Perondini, 1980), indicating that this is the primitive situation. *Drosophila* has retained some degree of non-autonomy as demonstrated by XX cells that initiate, but do not complete the spermatogenic pathway when transplanted into male hosts (Steinmann-Zwicky et al., 1989). Furthermore, at least in *Musca*, there is no dosage compensation so that there is no basis for sex-specific lethality. A temperature-sensitive mutation in *M* or *F* would create a new fertile strain with environmental sex determination, adding yet another variant to the already existing sex-determining "systems" within this single species (Table 1). We expect other Diptera that have no dosage compensation and whose germline completely follows the sexual pathway of the soma to display the same variability as *Musca* and to evolve new sex-determining systems equally easily. No such future is in sight for *Drosophila*.

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introduce the latest and detailled information. This is reflected by the choice of references, and we apologize to all authors whose work is not cited.

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