Sex comes in from the cold: the integration of sex and pattern

Audrey E. Christiansen, Eric L. Keisman, Shaad M. Ahmad and Bruce S. Baker

There has recently been a revolution in our understanding of how the *Drosophila* sex-determination hierarchy generates somatic sexual dimorphism. Most significantly, the sex hierarchy has been shown to modulate the activities of well-known signaling molecules (FGF, Wnt and TGFβ proteins) and transcription factors (BAB and DAC) to direct various sex-specific aspects of growth and differentiation. As some of the genes encoding these proteins are also the targets of Hox gene action, these and other findings are revealing the levels at which the sex determination and Hox patterning pathways are integrated to control growth, morphogenesis and differentiation.

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Audrey E. Christiansen
Eric L. Keisman
Bruce S. Baker*
Dept Biological Sciences, Stanford University, Stanford, CA 94305, USA.
*e-mail: bbaker@cmgm.stanford.edu

Shaad M. Ahmad
Dept Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305, USA.

A single multibranched regulatory hierarchy controls all somatic sexual differences in *Drosophila melanogaster* [1–3] (Fig. 1). The three branches of the hierarchy govern X chromosome dosage compensation, most somatic sexual differentiation (including female sexual behavior), and male sexual behavior. The initial steps in somatic sex determination assess the X chromosome:autosome ratio and establish sex by setting the activity of *Sex lethal* (*Sxl*) to 'on' in females (XX flies) and 'off' in males (XY flies). SXL turns off dosage compensation in females [4,5]. In addition, the presence or absence of SXL triggers cascades of sex-specific alternative mRNA splicing events that lead to the production of sex-specific *doublesex* (*dsx*) and *fruitless* (*fru*) products. The *fru* branch of the sex-determination hierarchy is responsible for the CNS-dependent aspects of male courtship behavior [6].

The *dsx* branch of the hierarchy is responsible for somatic sexual differentiation outside of the CNS [7], as well as several aspects of somatic sexual differentiation that might involve the CNS [8,9]. Both the male- and female-specific *dsx* mRNAs encode zinc finger proteins, DSX^M and DSX^F, respectively, which have identical DNA-binding domains but different carboxy termini [10,11]. The *dsx* gene is considered the last sex-determination regulatory gene in its branch of the hierarchy because its proteins bind to and directly regulate the transcription of two of the genes encoding terminal sexual differentiation proteins, the *Yolk protein-1* and *Yolk protein-2* (*Yp*) genes [10,12].

We focus on recent advances in two areas: (1) how the products of the regulatory genes at the bottom of the *dsx* branch of the sex hierarchy function together to bring about sexual development; and (2) the identification of the genes through which *dsx* acts to produce sexual dimorphism. The latter findings radically change our view of regulation by *dsx*, reconciling previously divergent views of *dsx* action.

Interactions of regulatory genes at the bottom of the hierarchy

In females, *dsx* acts with two other genes, *hermaphrodite* (*her*) [13–16] and *intersex* (*ix*) [17,18] to control sexual development. The *her* gene encodes a zinc finger protein [16], whereas *ix* encodes a protein that has no obvious DNA-binding domain but has homology to a region that functions as a transcriptional activator in several mammalian proteins that function as transcriptional activators [18], suggesting that *ix* might be a transcriptional activator. As described below, both *her* and *ix* are expressed sex-nonspecifically [16,18,19]. However, mutations in either *her* or *ix* are almost entirely (*her*) or entirely (*ix*) female-specific in their phenotypic effects [14–18], producing intersexual phenotypes similar to those produced by loss of *dsx* function in females. Both genetic and molecular data suggest that there are different reasons for the female-specificity of the sex-determination functions of *ix* and *her*.

Recent findings establish that the IX protein is an obligate partner with DSX^F. Genetic data show that IX and DSX^F function together to control all aspects of sexual differentiation examined; when either *dsx* or *ix* is mutant, the effect of removing the other gene is negligible [18,19]. The IX protein activates transcription of the *Yp* genes in females; this activation is dependent on a short region in the enhancer of the *Yp* genes that contains the DSX DNA-binding sites [18]. Physical interaction of the IX and DSX^F proteins was first suggested by the finding of a cold-sensitive intersexual phenotype in diplo-X flies that were simultaneously heterozygous for specific mutant alleles of *ix* and *dsx* [20,21]. Such cold-sensitive nonallelic noncomplementation is often indicative of protein–protein interactions [22,23]. Physical interactions of IX and DSX^F have been recently demonstrated by yeast two-hybrid, co-immunoprecipitation and gel-shift assays [18]. These findings indicate that the IX and DSX^F proteins function together in a complex controlling sexual differentiation in females.

Functional interactions between HER and DSX^F are more complicated: in some sexually dimorphic tissues, HER, like IX, is dependent on DSX^F for its effects, whereas in other tissues HER and DSX^F act independently and in parallel, because there is an additive effect of mutations in these genes on sexual differentiation in these tissues [15]. Consistent with the finding that HER and DSX^F promote *Yp* gene expression

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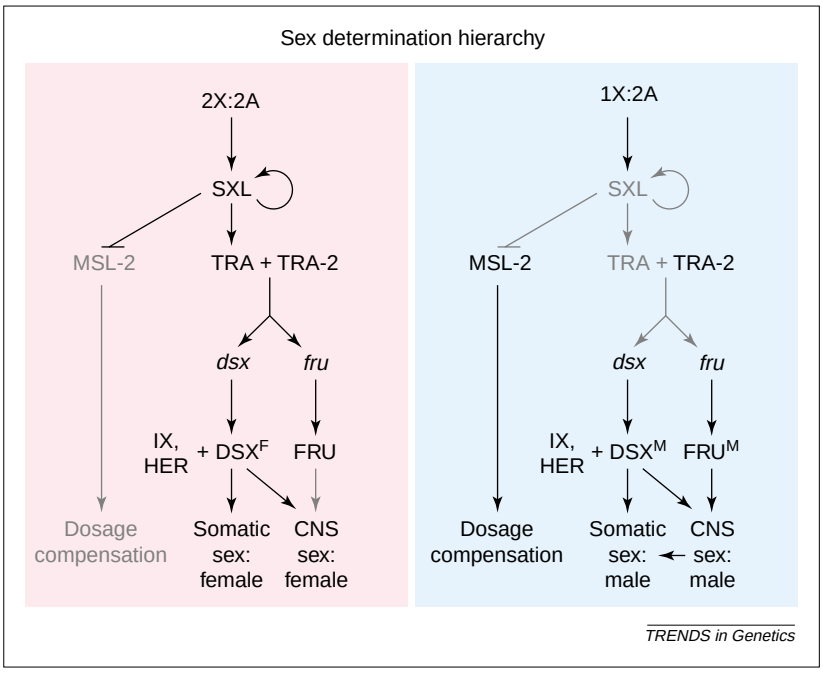

Fig. 1. Sex-determination hierarchy. Black indicates active; gray indicates inactive or nonfunctional. Females (pink background): the *Sxl* gene is maintained on in females by a positive autoregulatory loop. In addition to regulating the processing of its own pre-mRNA the SXL protein also functions in females to control the activity of two subservient branches to this hierarchy. One branch governs somatic sexual differentiation [1–3], and the other dosage compensation [72,73]. SXL prevents dosage compensation in females by blocking the translation of *msl-2* mRNA [4,5]. SXL regulates female somatic sexual differentiation by controlling *transformer* (*tra*) pre-mRNA splicing so as to generate a female-specific mRNA encoding the TRA protein. In females, the *tra* and the constitutively expressed *transformer-2* (*tra-2*) genes function together to control all aspects of somatic sexual differentiation. After this point, the somatic sex-determination hierarchy splits, with *tra* and *tra-2* controlling two parallel branches of the sex-determination hierarchy. Immediately below *tra* and *tra-2* in one branch is the *doublesex* (*dsx*) gene and in the other branch is the *fruitless* (*fru*) gene [6]. In females, TRA and TRA-2 regulate the splicing of *dsx* and *fru* pre-mRNAs to generate female-specific *dsx* and *fru* mRNAs. The female-specific *fru* mRNAs are not translated to sex-specific products. In the case of *dsx*, both the male- and female-specific *dsx* mRNAs encode zinc finger proteins that have identical DNA-binding domains but different carboxy termini. In females, *dsx* functions with the *intersex* (*ix*) [17] and *hermaphrodite* (*her*) genes [13–16]. The *dsx* branch is required for all aspects of somatic sexual differentiation outside of the central nervous system (CNS) and some aspects of sexual differentiation that could involve the CNS, possibly including some aspects of sex-specific behavior [9,19]. In males (blue background), default splicing of *Sxl* pre-mRNA results in the inclusion of a male-specific exon containing stop codons, and no protein is made. In the absence of SXL protein, *msl-2* mRNA is translated and dosage compensation occurs. In addition, the *tra* pre-mRNA is spliced by a default pathway, which results in the inclusion of premature stop codons that prevent TRA protein production. Because TRA is absent, *dsx* and *fru* pre-mRNAs are spliced to form default, male-specific *dsx* and *fru* mRNAs. The male-specific *fru* mRNAs are translated to produce BTB-zinc finger transcription factors. The *fru* branch of the sex-determination hierarchy is responsible for nearly all CNS-dependent aspects of sexual differentiation, including male courtship behavior and the sex-specific differentiation of the muscle of Lawrence [74].

in an independent manner, the effect of HER on *Yp* gene expression is mediated through DNA sequences that do not contain the DSX-binding sites [15]. In those tissues where HER and DSX^F act independently, the apparent female specificity of HER is achieved by DSX^M overriding HER in males [16]. For example, HER promotes *Yp* gene expression independent of sex, but in males this effect is countermanded by DSX^M-controlled repression of *Yp* transcription.

On the nature of sexual dimorphism in *Drosophila*

Because investigators of the sex hierarchy have previously focused on regulatory gene interactions, and because the aspects of sexual differentiation most evident to the general *Drosophila* researcher are sometimes viewed as relatively minor embellishments on the body plan (remove a little pigment here, sharpen up the bristles in that row a bit, add some yolk protein, *etc.*, and *Voila*! – you have turned a male into a female), there has been a tendency to consider sex as a relatively superficial embellishment on more basic developmental programs that lay down the body plan and specify development within fields of cells [24,25]. Yet a deeper look at sexual differentiation in flies shows that it also involves the control of growth at an organismal level (females are bigger than males, Fig. 2a,b) and profoundly affects patterning and growth at the level of imaginal discs (the patterns of growth and morphogenesis of the genital disc in males and females are very different, Fig. 2e,f). Thus the sex-determination hierarchy must control not only the expression of terminal differentiation genes in sexually dimorphic tissues, but also the patterns of growth and morphogenesis that build those tissues. As such, the sex-determination hierarchy controls a complex array of developmental processes that are similar to those controlled by other major developmental regulatory genes, like the homeotic (Hox) genes.

Regulation of sexual dimorphism: early studies

Early studies of how sexual differentiation is controlled by the *dsx* branch of the hierarchy led to two significant generalizations [7]. First, *dsx* can act both negatively [26] and positively [8,12] to regulate various target genes. For example, transcription of the *Yp1* gene is repressed by the DSX^M protein, but activated by the DSX^F protein [12]. Second, genes expressed sex-specifically are controlled by the hierarchy in either of two ways. Some terminal differentiation genes are direct targets of the hierarchy, and require the continuous action of the hierarchy, even in adults, for their expression (e.g. the *Yp* genes in the fat body [27]). Other terminal differentiation genes appear to be regulated indirectly by the hierarchy; the decision to express (or not) such genes is irreversibly made during development, long before the time they are expressed. For example, transcription of the male-specific gene *Acp95EF* begins in the late pupal period, but it is in the late larval period that the hierarchy determines whether *Acp95EF* will be transcribed [28,29].

DiBenedetto *et al.* [28] suggested that these differences in regulation reflect two different mechanisms: (1) in cases like the *Yp* genes, the sex hierarchy directly controls transcription of a terminal sexual differentiation gene in a tissue that is present in both sexes; and (2) in cases like *Acp95EF*, the sex hierarchy controls whether a tissue that is present in only one sex is formed, with the subsequent patterns of gene expression in that sex-specific tissue being brought about as part of a tissue differentiation program rather than being directly controlled by the sex hierarchy. For example, the *Yp* genes are expressed in a tissue (fat body) common to the two sexes, and are direct transcriptional targets of the DSX proteins, whereas *Acp95EF* is expressed only in

Fig. 2. Sexual dimorphism in *Drosophila melanogaster*. The effects of the sex-determination hierarchy are apparent at different stages and in different tissues of the fly. Female adult flies (a) are larger than male adult flies (b). In addition, the pigmentation of the abdomens is different between the sexes, with males having fully pigmented (dark) terminal segments. (c) In females, the last two transverse rows of bristles on the first tarsus of the foreleg develop like the other rows of tarsal bristles, whereas in males (d) these rows of bristles are rotated and thickened to form the sex comb. Confocal images of mature genital discs – (e) female; (f) male – reveal the sexual dimorphism apparent in the growth of these discs using a nuclear localized green-fluorescent protein (blue) driven by an imaginal tissue-specific enhancer trap line ($esg^{GAL4.8}$) [42] and propidium iodide (red) as a counterstain to show the mesodermal cells. In the female genital disc, the male primordium (top) does not proliferate extensively and remains a thin layer whereas the female primordium proliferates; the associated mesodermal cells form a pair of central clusters (arrowhead in e) that are surrounded by the female primordium. In the male genital disc, the male primordium (top half) proliferates extensively and recruits mesodermal cells, which form two clusters in the male primordium (red, arrowhead in f) [64]; the female primordium (bottom middle) does not proliferate as extensively whereas the anal primordium (bottom corners and out of plane) proliferates in both sexes (see Fig. 3 for comparison). These imaginal discs generate the sex-specific genitalia and analia (see Laugé [32] for descriptions of internal and external structures). In (g), the female external genitalia have several obvious sexually dimorphic features, including the twin rows of thorn bristles (arrow) that line the vaginal opening. One derivative of the anal primordium, the anal plates, are also sexually dimorphic with the two anal plates oriented dorsally and ventrally in females (not shown). (h) The male external genitalia are far more complex. The male anal plates (arrow in h) are oriented laterally. Just anterior to the genitalia (arrowheads in g and h) is the 6th sternite, which is covered with bristles in females but is bare in males. The formation of these bristles requires DSX^F, *Abd-B*, and *bab* [55,59].

the accessory glands, or paragonia, of the male internal genitalia, tissues that are present in only one sex. In the case of *Acp95EF*, the role of the *dsx* branch of the hierarchy would be to control whether or not paragonia will form. Consistent with this expectation, the time at which the sex hierarchy determines whether *Acp95EF* will be expressed is the same time at which it specifies paragonia formation [29]. Until recently, there were no clues as to the identity of the genes that were needed downstream of *dsx* to account for how sex-specific tissues like paragonia were built.

The recent advances in understanding how *dsx* regulates sexual differentiation have revealed how sex-specific tissues like paragonia are built. As many of these advances come from work on the genital imaginal disc we will briefly discuss genital disc development.

The *dsx* gene in the genital disc and the fates of the repressed genital primordia

The genital disc gives rise to the genitalia and analia (Fig. 2g,h). By contrast to most imaginal discs, which are essentially two-dimensional [30], the genital disc is a three-dimensional structure with distinct dorsal and ventral epithelia, which generate the different adult genital and anal structures [31,32] (Fig. 3). The genital disc is strikingly sexually dimorphic (Fig. 2e,f). The three primordia – female genital, male genital, and anal – that make up the genital disc originate from cells derived from three embryonic tail segments, A8, A9 and A10, respectively [31,33] (Fig. 3a).

In the genital disc, the two alternative fates of forming male versus female genitalia were believed to be embodied in two distinct cell populations, the male and female genital primordia, that are specified in every embryo [33–36]. In each fly, the sexually appropriate genital primordium was thought to proliferate and differentiate, whereas the inappropriate genital primordium was believed to proliferate little, and not contribute to the adult. The third primordium of the genital disc, the anal primordium, is like other embryonic primordia in that its cells can adopt one of two alternative fates, either male or female. This view of the development of the male and female genital primordia was important for the field because of what it implied about the role of the sex hierarchy in directing the development of the genitalia. It appeared that the only function required of the *dsx* proteins for genital development was to restrict the development of the inappropriate genital primordium. This view arose from the observation that in *dsx*-null mutants, both primordia develop [26,37]. The actual fate of the primordium that developed was thought to be specified by the segmental identities of the genital primordia (A8 or A9) provided by the Hox genes of the bithorax complex [38–41].

This view of the fates of the inappropriate genital primordia has confounded thinking about sex determination in general, and, it turns out, misdirected thinking with respect to sex determination in the genital disc in particular. These findings generated an ambiguity as to the basic role of the sex hierarchy. Some data suggested the hierarchy had an instructive role in directing differentiation (e.g. *dsx* turning *Yp* genes on in females and off in males), whereas other data suggested that *dsx* functioned merely in a restrictive manner (e.g. in preventing growth of the inappropriate genital primordium). This ambiguity as to how *dsx* functioned has been resolved with the discovery that one aspect of

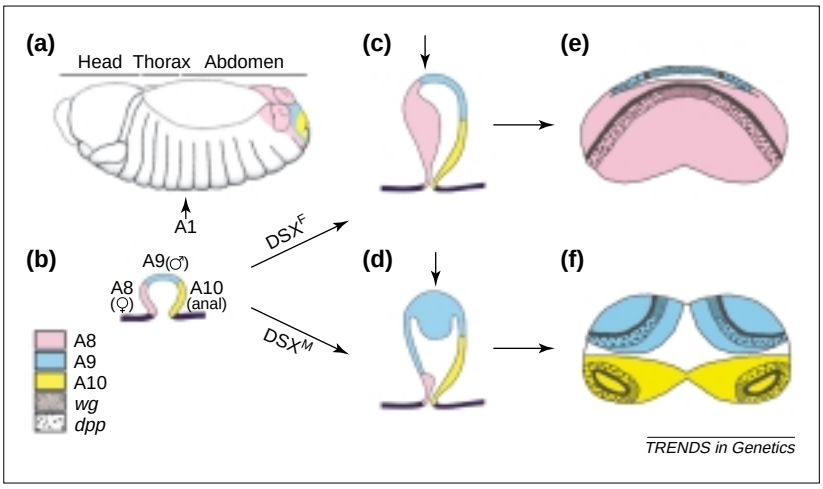

Fig. 3. Growth of the genital imaginal disc. (a) Embryo, stage 13 (modified, with permission from Ref. [75] © 1993 CSHL press). The three most posterior abdominal segments, A8 (pink), A9 (blue) and A10 (yellow), with the anal pad form the posterior end of the embryo. The first abdominal segment, A1, is indicated. Cells from A8, A9 and A10 form the genital disc precursor cell (GDPC) cluster in the ventral epidermis. (b) These cells invaginate as a cluster at the end of embryogenesis to form the genital imaginal disc. During larval development, the sex-specific DSX proteins regulate the proliferation of the genital disc in a segment-specific manner, generating significant growth in the female (in (c) A8) or male (in (d) A9) primordium as shown in cross-section. Arrows in (c) and (d) indicate the planes used to draw (e) and (f). *wg* (heavy stippling) and *dpp* (light stippling), shown overlaid on female (e) and male (f) genital discs (modified from Chen and Baker [43]), are expressed in the anterior compartment along the posterior compartment boundary; the thickened line indicates the posterior edge of the anterior–posterior (A/P) organizer in each of the three segments.

the long-accepted view of the development of the genital primordia was incorrect [42].

The key finding that realigned thinking about the role of the sex-determination hierarchy in the genital disc was the discovery that the male and female genital primordia have defined fates, and give rise to parts of the adult in both sexes [42]. There are no 'repressed' genital primordia. Using the expression patterns of genes such as *engrailed* and *wingless* as markers to track the different genital primordia during metamorphosis, these experiments showed that each primordium gives rise to specific adult structures in each sex. In males, the male primordium forms the male genitalia, whereas in the female it forms a pair of female-specific accessory glands, the parovaria, and part of the uterine wall. In females, the female primordium gives rise to the female genitalia, whereas in the male it produces a miniature eighth tergite. These findings show that in the one case where *dsx* had been inferred to have only a restrictive function, it has an instructive function – directing the female genital primordium to form either female genitalia or an eighth tergite, and the male genital primordium to produce either male genitalia or the parovaria. Consequently, *dsx* acts instructively in all its known functions.

Regulation of sexual dimorphism: recent studies

The past three years have seen the identification of five of the downstream genes through which *dsx* and its partners act. As these five genes encode well-known developmental regulatory proteins, the finding that sex modulates the activities of these genes has provided substantial insight into how sexual dimorphism is generated at the tissue and cellular level, as well as the levels at which information from the sex hierarchy and other patterning hierarchies is integrated.

DSX and the Hox genes specify patterns of cell proliferation in the genital disc

In genital discs, like the thoracic discs, the cells at the anterior–posterior (A/P) compartment borders that express *wingless* (*wg*) and *decapentaplegic* (*dpp*) function as organizers. These A/P organizers control growth and patterning throughout the disc [43–48] (Fig. 3e,f). Hox genes functioning in the cells of A/P organizers modulate *wg* and *dpp* signaling [44,49–51]. That the sex hierarchy might also modulate the A/P organizer's function in the genital disc was suggested by the behavior of clones of female cells in the male genital primordium [48]. Because the female genital primordium does not grow much in males, it was anticipated that female clones in the male primordium would grow slowly at best. However, many such clones continued to proliferate well, although occasionally such clones did disrupt growth [42]. The dichotomous behavior of these clones led to the suggestion that growth in the genital primordia is controlled nonautonomously from within an organizing region [48]. Clones that grow normally would lie outside this organizing region, whereas those that cause reductions would intersect it. Obvious candidates for the growth-organizing regions are the A/P organizers of each of the three primordia of the genital disc.

Manipulating the sex of defined sets of cells within the genital disc using the GAL-4/UAS system shows that the major factor controlling the growth of the genital primordia is the sex of the cells in the A/P organizers, not the sex of individual cells throughout the genital disc [42]. When the cells of the A/P organizers are feminized in a male disc, or masculinized in a female disc, both genital primordia respond by switching to growth patterns that reflect the sex of the organizers. These changes are profound: a prepupal ('mature') genital disc of one chromosomal sex in which the sex of the A/P organizers has been changed is difficult to distinguish morphologically from a wild-type disc of the opposite sex. When the sex of posterior compartment cells is genetically altered (as a control), there is no major change in disc morphology, suggesting that these posterior compartment cells continue to grow normally under the influence of the unaffected A/P organizers. Furthermore, changing the sex of cells in the genital primordia can change the expression of *wg* and *dpp* in these cells [44]. These data strongly suggest that *dsx* acts within this organizing region to control the expression of *wg* and *dpp* and thereby dictate sex-specific patterns of growth within the genital disc [36].

The different segmental identities of the primordia must also have major roles in modulating signaling from the A/P organizers given the profound differences that sex has on the growth of the A8-, A9- and A10-derived primordia. These three posterior

segment-derived primordia are specified by a segment-specific level and mixture of homeotic gene expression of *abdominal-A* (*abd-A*), *Abdominal-B* (*Abd-B*) and *caudal* (*cad*) [52–54].

DSX and the Hox genes function together to specify sex-specific patterns of abdominal pigmentation

Male *Drosophila melanogaster* have darkly pigmented 5th and 6th abdominal segments (Fig. 2b), a feature that females lack (Fig. 2a). These sexually dimorphic abdominal pigmentation patterns are brought about by a segment- and sex-specific expression pattern of *bric à brac* (*bab*) [55,56]. The *bab* locus contains two genes, *bab* and *bab-II*, that encode BTB zinc finger transcription factors [55,57–59]. Analysis of the phenotypes produced by both loss-of-function *bab* mutants and ectopic *bab* expression showed that *bab* is capable of repressing pigmentation in abdominal segments A2–A6 in both sexes. However, in the wild type, *bab* is expressed in A2–A6 in females but only in A2–A4 in males. The absence of *bab* expression in A5 and A6 in males correlates with male-specific pigmentation of these segments.

The sex-specific and segment-specific deployment of *bab* is brought about by the joint action of *Abd-B* and *dsx*. Analysis of the effects of loss-of-function *Abd-B* mutants and ectopic *Abd-B* expression show that *Abd-B* functions in the wild type to repress *bab* expression in A5 and A6 in both sexes, allowing pigmentation in these segments. Analysis of *dsx* loss-of-function mutants demonstrates that, in females, DSX^F counteracts the repression of *bab* by *Abd-B*, thus restoring *bab* expression in A5 and A6 in females and preventing pigmentation of these segments [55]. As such, *dsx*, together with *Abd-B*, results in *bab* being repressed only in the A5 and A6 segments in males, generating the observed sexually dimorphic patterns of abdominal pigmentation.

In both *dsx*-null females and males A5 pigmentation does not extend quite as far anteriorly as it does in wild-type males, suggesting that in males DSX^M acts to ensure complete pigmentation of this segment [26,55,60]. In addition, the *Abd-B* and *abd-A* genes may, to a lesser extent, regulate abdominal pigmentation through a *bab*-independent pathway [55].

Sex-specific regulation of FGF signaling directs formation of a male-specific tissue

The *branchless* (*bnl*) gene, which encodes a fibroblast growth factor (FGF) [61], and the *breathless* (*btl*) gene, which encodes an FGF receptor [62,63], are each expressed in two bilaterally symmetrical and adjacent groups of cells in the mature male genital disc, but not in the female genital disc [64]. The *btl*-expressing cells are not originally part of the male disc; instead, they are actively recruited into it during late larval development. Expression of FGF in the ectoderm-derived cells of the male genital disc induces the *btl*-encoded FGF receptor-expressing mesodermal cells to migrate into the male discs (arrowhead in Fig. 2f).

The male-specific deployment of FGF is brought about by *dsx*: *bnl* is repressed in a cell-autonomous manner in the female genital disc by the DSX^F protein, restricting *bnl*-expressing cells to the male genital disc [64]. Indeed, *bnl* could be a direct target of *dsx* because the 5′ untranscribed region of *bnl* contains two clusters of putative DSX-binding sites similar to those in the regulatory region of the *Yp* genes [64].

Unlike the other cells in the genital disc, which are derived from the embryonic ectoderm, the recruited *btl*-expressing cells originate from the mesoderm and initially express the mesodermal marker *twist* [64]. After being recruited into the disc during the late third instar, these cells define a novel third compartment that is clonally distinct from the anterior and posterior compartments. Subsequently, the *btl*-expressing cells become epithelial, losing *twist* expression and expressing CORACLE, a component of septate junctions in epithelia. These cells give rise to the paragonia and vas deferens, major parts of the internal male genitalia. Interestingly, in the absence of *dsx* function, *bnl* is expressed in two groups of cells in both the male and female primordia of the genital disc, and *btl*-expressing cells are recruited into the genital primordia at all four locations where *bnl* is expressed [64]. Moreover, *dsx* mutant discs frequently give rise to four paragonia, rather than the two present in wild-type males [65]. These observations lead to the rather surprising conclusion that one way the sex hierarchy functions to generate sex-specific tissues is to repress the formation of a tissue in one sex.

The dsx gene modulates the response to wg and dpp signaling in the genital disc

Signaling by *wg* and *dpp*, in addition to governing cell proliferation in imaginal discs, specifies pattern in imaginal discs [66–68]. As such, the sex-specific differentiation of the genital (and anal) primordia may entail the sex-specific interpretation of *wg* and *dpp* signals by receiving cells. Bearing out this prediction, the *dachshund* (*dac*) gene is expressed in a sex-specific pattern in the genital disc under the control of *dsx* [48,69]. In the male primordium of an XY genital disc, *wg* represses *dac* whereas *dpp* activates it, so *dac* overlays the *dpp* expression domain (Fig. 3f). In the female primordium of an XX genital disc, this relationship is inverted: *wg* activates *dac* and *dpp* represses it, so *dac* overlays the *wg* expression domain (Fig. 3e). In sexually mosaic discs, *dac* expression in response to *wg* and *dpp* varies in perfect correlation with the sex of the responding cell, demonstrating that this effect is under the cell-autonomous control of the sex-determination pathway [48].

Sex-specific *dac* expression correlates with a sex-specific requirement for *dac* function in genital disc derivatives: *dac* is needed to construct properly the internal ducts connecting the spermathecae and parovaria to the uterus in females and to construct the claspers – a set of cuticular structures – in males [48]. Thus, the sex-specific deployment of

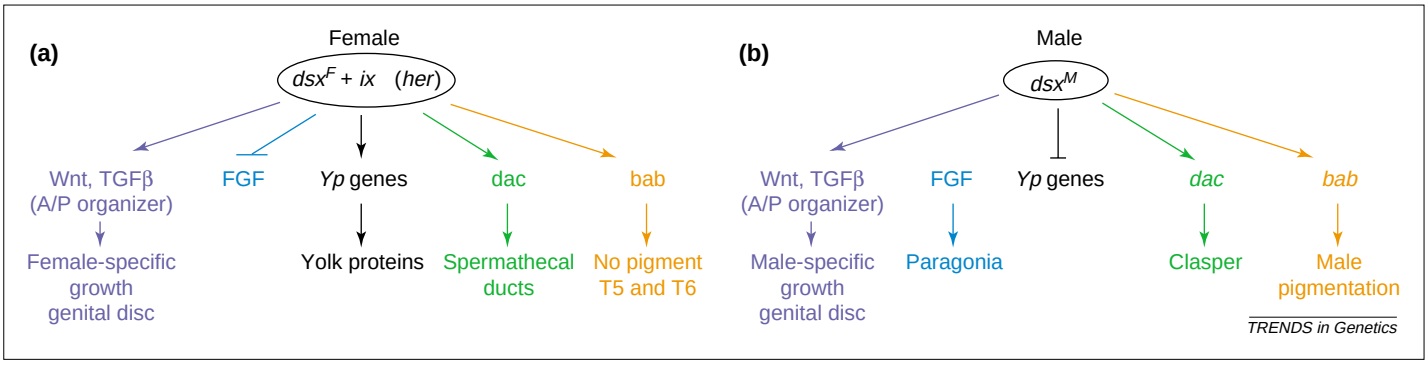

Fig. 4. The role of *dsx*, past and present. Until recently, the known targets of *dsx* (black) were limited to the *Yp* genes, which are sex-specifically activated by DSX^F in females and repressed by DSX^M in males. Note that DSX^F functions with IX and either with or in parallel to HER in females (see text) to regulate sex-specific development. Recent work has identified five additional targets of the sex hierarchy (colored). *dsx* regulates the sex-specific growth of the genital disc by the disc's anterior–posterior (A/P) organizer [42] (purple). *dsx* regulates the sex-specific expression of a fibroblast growth factor (FGF) in the genital disc, leading to the development of the paragonia in males [64] (blue). *dsx* alters the regulation of *dac* to produce both sex-specific patterns of *dac* expression in the genital disc [44,48] and subsequent sex-specific structures in the genitalia [48] (green). *dsx* sex-specifically modulates the repression of *bab* to produce sex-specific pigmentation patterns on the abdomen [55] (orange).

dac leads to its functioning in the development of nonhomologous positions of the male and female genital discs.

Conclusion

The last three years have seen a revolution – both conceptually and factually – in our understanding of how the *dsx* branch of the sex hierarchy functions to control sexual development. One key conceptual change was the finding that the manner in which *dsx* modulates development can be explained in a unitary model in which *dsx* is instructive in all its known functions. In addition, our understanding of what the sex hierarchy does has been substantially broadened by demonstrations that this hierarchy controls not only the differentiation of sex-specific structures, but also patterns of cell growth in the genital imaginal disc, directs sex-specific cell migration, and modulates the responses of cells to key organizing signals such as *dpp* (TGFβ) and *wg* (Wnt) (Fig. 4).

The sex-determination hierarchy brings about many aspects of sexual development by sex-specifically modulating the activities of well-known generic cell–cell signaling molecules and transcription factors. This highlights the question of how specificity is achieved. Because the sex hierarchy modulates the activities of these downstream genes in a segment/tissue-specific manner, there are two extreme, and not necessarily mutually exclusive, ways that specificity might be achieved: (1) the sex hierarchy might be able to modulate the activities of these downstream genes only when the appropriate array of other segment/tissue-specific transcription factors is present in cells; (2) one or more of the proteins encoded by the sex hierarchy might be subject to modification such that its activity is spatially modulated.

Finally, these recent studies provide substantial insight into the levels at which information from the sex hierarchy is integrated with information from the patterning hierarchies that build the basic body plan. Several independent results lead to the rather surprising conclusion that *dsx*, in a variety of circumstances, is functioning at the same level as the Hox genes and it is through the joint regulation of downstream signaling molecules and transcription factors that information from these hierarchies is integrated (reviewed in Refs 36,56,70). Previous studies noted that the functions of *dsx* and the Hox genes have many formal similarities [26,71]. These recent findings suggest there is a mechanistic basis for these similarities: one function of the DSX proteins is as sex-specific regulatory partners of the Hox genes in sexually dimorphic tissues. However, this role is not the only function of the sex hierarchy, because it also directly regulates the *Yp* genes. All told, the functioning of the sex-determination hierarchy is integrated at several different levels in the development of a fly.

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