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Doublesex Surprises

Minireview

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Morphologically distinct males and females are observed throughout the animal kingdom. The developmental and molecular events leading to the establishment of sexual dimorphism are not only fascinating problems for developmental biologists, but are also essential to the survival of the species. How the genetic sex of an animal regulates the developmental events that determine the sexual identity as well as control the size and shape of specific organs is a fundamental problem. One could formulate two hypotheses to explain the origins of sexual structures. First, since male and female reproductive structures are different, it follows that they might arise from two distinct populations of precursor cells. Which population is chosen for further development would be dependent on the genetic sex of the animal. Alternatively, both male and female structures might develop from common precursor tissues that differentiate in response to signals determined by the genetic sex of the animal. Recent studies from *Drosophila* provide new, and somewhat surprising, insights into this interesting problem.

The "Classic" Model of Sexually Dimorphic Development of the Genital Disc

During metamorphosis in *Drosophila*, essentially all larval tissues are hydrolyzed and the adult body is assembled via the coordinated differentiation and integration of imaginal disc cells. The precursor cells of the imaginal discs are specified during embryogenesis, proliferate and form an epithelial sac-like structure during larval stages, and undergo a complex morphogenesis during pupal stages. The genital imaginal disc gives rise to the terminalia, which consist of both internal and external genital and anal structures, excluding the gonads (Bryant, 1978). Interestingly, the development of the genital disc is completely sexually dimorphic in that all adult structures derived from it are different in males and females.

Previous studies indicated that the anlage of the genital disc of both sexes is composed of three fields of cells or primordia: the female genital primordium (FGP), the male genital primordium (MGP), and the anal primordium (AP). These primordia arise from three different embryonic abdominal segments, A8, A9, and A10, respectively. The AP grows in both sexes and develops into either the male or female analia. According to the "classic model," only the genital primordium that corresponds to the genetic sex grows, while the inappropriate primordium is repressed (Figure 1). In this model, it is thought that both MGP and FGP are derived from different groups of embryonic cells (Nothiger et al., 1977; Schupbach et al., 1978). Further, in each animal, development of the inappropriate genital primordium is repressed by the sex-specific products of the *doublesex* (*dsx*) gene (Baker and Ridge, 1980).

The *dsx* gene encodes two zinc finger proteins, Dsx^m in males and Dsx^f in females. With the exception of the central nervous system, the Dsx proteins determine the sexual fate (male or female) of each somatic cell of the fly. A splicing factor, encoded by the *transformer* (*tra*) gene, is active only in female cells. Along with the product of the *transformer 2* gene, Tra splices the *dsx* message into the female form, dsx^f. In male cells, absence of the Tra splicing complex results, by default, in the splicing of the *dsx* message into the male form dsx^m (reviewed in Cline and Meyer, 1996).

The view that the MGP and FGP have separate cellular origins stems from phenotypic analyses of mosaics comprised of both male and female cells. In *Drosophila*, sex is determined by the ratio of X chromosomes to sets of autosomes. There is no hormonal regulation of somatic cell sexual development and each cell of the body directly controls its own sexual fate. Thus, random loss of one X chromosome in an XX (female) embryo during embryonic divisions produces a mosaic in which parts of the body carrying two X chromosomes adopt the female identity, while the remaining parts have only one X chromosome and develop male characteristics. The pivotal evidence suggesting separate MGP and FGP was found in animals in which the mosaic border separated the two primordia. In this case, both the resulting XX female primordium and XO male primordium developed, producing a fly with two complete, or nearly complete, sets of genitalia (Nothiger et al., 1977; Schupbach et al., 1978). The hypothesis that only the genital primordium that corresponds to the genetic sex grows, while the inappropriate primordium is repressed, is corroborated by experiments where defined fragments from the genital disc were cultured and analyzed as to the derivatives they produced. These data suggested there were regions in the genital disc in each sex that did not appear to differentiate into adult structures, and these regions were inferred to be the repressed primordia of the inappropriate sex (Epper and Nothiger, 1982).

This model fits well with genetic studies suggesting that sex-specific products from the gene *dsx* autonomously repress the development of the inappropriate genital primordium. In *dsx* mutant animals, in which both Dsx^m and Dsx^f functions are removed, both the MGP and FGP develop (Baker and Ridge, 1980). These authors concluded that in males, Dsx^m represses the female primordium and allows the development of the male genitalia. Conversely, in females, Dsx^f has the reciprocal effect. Thus, it appeared that *dsx* functioned as a control gene that repressed development of the inappropriate primor-

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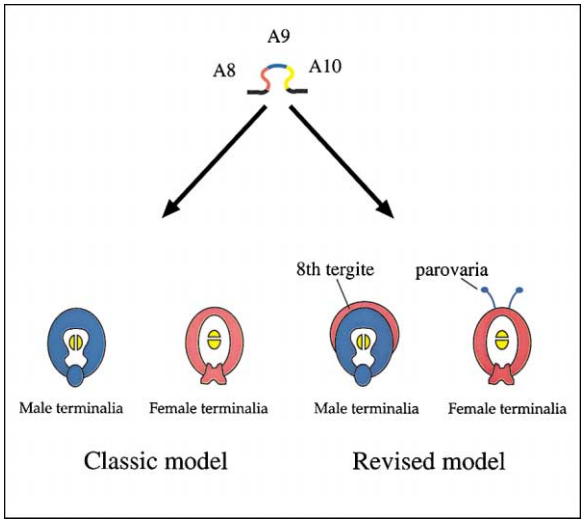

Figure 1. Classic and Revised Models of Genital Disc Development

The difference between the classic and revised models consist in the type of adult derivatives derived from the primordia of the 8th and 9th abdominal segments (A8 and A9).

dium, allowing, by default, the appropriate primordium to develop.

A Revised Model of Sexual Dimorphic Development of the Genital Disc

The classic model of development of the genital disc needs to be substantially revised in light of new findings described in three recent reports (Keisman and Baker, 2001; Keisman et al., 2001; Sanchez et al., 2001). First, Keisman et al. (2001) demonstrate that in each sex, both the primordia of the A8 and A9 segment, previously known as FGP and MGP, respectively, contribute to defined adult structures. That is, the genetically inappropriate primordium is not repressed. Further, all three of these papers contribute to the finding that the *dsx* products, Dsx^f and Dsx^m, direct the sex-specific growth of the genital primordia by regulating both signaling from an organizer region, as well as the cellular responses to these signals throughout the disc.

Keisman et al. (2001) have conclusively demonstrated that neither the A8 nor the A9 primordia are repressed in the inappropriate sex. Using specific markers that label small groups of cells, they found that while in males, the A9 primordium gives rise to the male genitalia, in females, this primordium actually develops into accessory glands known as parovaria. Likewise, while the A8 primordium in females gives rise to the female genitalia, in males, this primordium develops into a little known eighth tergite, a piece of dorsal cuticle in the adult. Thus, instead of two distinct primordia giving rise to all male- and female-specific structures, the new revised model (Figure 1) proposes that both genital primordia contribute to the complement of genital structures in each sex.

What controls the sex-specific patterns of growth of the genital disc? An analysis of clones of female cells in the genital disc of genetically male flies, and of clones of male cells in the genital disc of genetically female flies, led Keisman and Baker (2001) to predict the existence of a growth organizer in each primordium. The growth

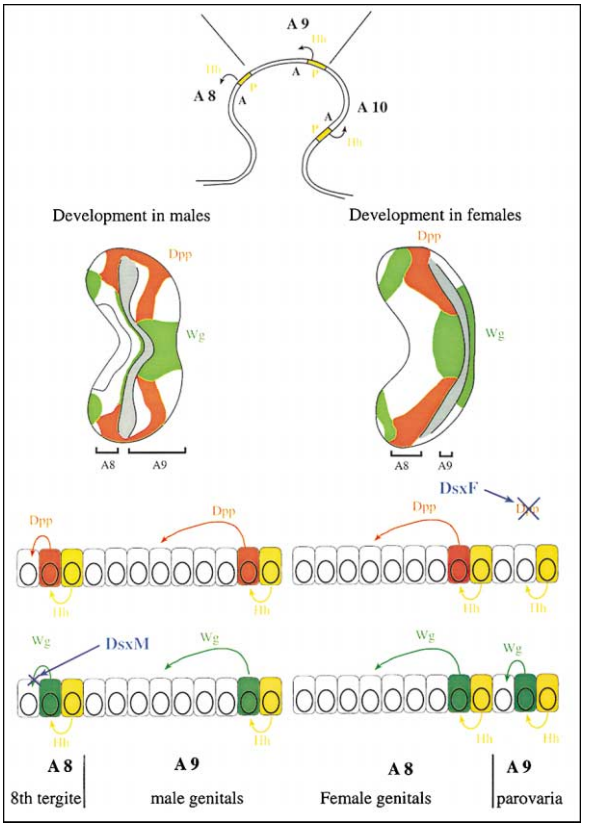

Figure 2. Signaling Pathways and Genital Disc Development

The genital disc is a compound disc containing the primordia of the female and male genitalia, and the analia, which are derived from abdominal segments A8, A9 and A10, respectively. Hh, expressed in the posterior (P) compartment of each segment determines the position of the organizer in the anterior part of the disc. A scheme of the male and female genital discs depicts the expression of dpp and the one of Wg in green. The gray area indicates the lumen of the discs. Note that *dpp* is not expressed in the female abdominal 9 segment. As in the leg disc, Dpp and Wg expression does not overlap. (For further details on the complex spatial organization of the *dpp* and *wg* expressing cells in the genital discs, see Sanchez et al., 2001 and Keisman et al., 2001) The action of DsxM and DsxF is shown below on each group of cells.

organizer would represent a group of cells from which emanate signals that act at a distance to regulate sex-specific patterns of proliferation. In these experiments, clones of cells of the inappropriate sex in some locations were found not to affect growth, whereas in other locations such clones profoundly affected growth, suggesting that growth in the genital primordium is controlled nonautonomously.

Based on studies of other imaginal discs, an excellent candidate for this organizing region is the Patched-expressing strip of cells located in the anterior compartment, adjacent to the A/P border. The cells giving rise to the A8 primordium and those giving rise to the A9 primordium are hypothesized to be controlled by a different organizer (Figure 2). To test this hypothesis; i.e., to determine whether differential growth of the A9 and A8 primordia involves sex-specific regulation of the two A/P organizers, Keisman et al. (2001) altered the genetic sex of the A/P organizer regions. To feminize the A/P

organizers present in the male genital disc, they ectopically expressed the tra gene using Patched-Gal4. In these flies, the morphology of the entire genital disc was dramatically changed, giving it the appearance of a female disc. Conversely, to masculinize the A/P organizers of the female genital disc, they used Patched-Gal4 to express a tra2 inverted repeat construct that blocks the function of tra2 through the mechanism of double-stranded RNA interference. In these flies, the masculinized organizers influenced the morphology of the entire genital disc, giving it the appearance of a male disc. Together, these results indicate that, in both cases, the sex of the cells comprising the organizers determines the overall growth and morphology of the entire genital disc.

The results of Keisman et al. (2001) suggest that there are a number of intersections between the circuitry that controls sexual identity and the circuitry that regulates growth and pattern formation in the genital disc. If the sex determination pathway regulates the A/P organizer, it must do so by regulating the expression of the morphogen signals that emanate from it. And if the sex-determination pathway directs the differentiation of each genital primordia down a sex-specific pathway, it must also regulate the sex-specific interpretation of these morphogenetic signals in the receiving cells. A number of recent results (Sanchez et. al., 2001, Keisman and Baker, 2001) support this model.

In the recent studies, the authors examined the function of the secreted signaling molecules Hedgehog (Hh), Decapentaplegic (Dpp), and Wingless (Wg), which have been shown to organize pattern formation in other imaginal discs. The organization of the genital disc is reminiscent of the leg disc (Gorfinkiel et al., 1999), with Hh establishing an A/P organizer region from which Wg and Dpp signals emanate. In this A/P organizer, *wg* and *dpp* are expressed in abutting, nonoverlapping groups of cells. Subsequently, the morphogenetic activity of Wg and Dpp spreads to pattern the remaining disc. In the genital disc, the authors find that Dsx is able to control the activity of Dpp and Wg at multiple levels (Figure 2). In the female genital disc, Dsx^f acts in the A9 primordium to block the transcription of *dpp* induced by Hh, while in the male genital disc, Dsx^m modulates the response of genital precursor cells to the Wg signaling pathway. For example, in the A9 primordium of a male disc, Dpp activates expression of the *dachshund* (*dac*) and Wg inhibits it, while in the A9 primordium of a female disc, Dpp represses *dac* transcription and Wg activates it.

Collectively, the studies described above demonstrate that sexual dimorphism in the genital disc is controlled in two ways: growth is regulated by the sex-specific signaling from the A/P organizer, while determination and differentiation is controlled cell autonomously. Individually, each cell of the genital primordia "knows" whether it is male or female, and that information is used to respond appropriately to the sex-specific, modulated signals from the organizer regions.

Cooperation between Dsx and Homeotic Genes

These results indicate that the functions of Dsx during growth and differentiation of the genital disc are more complex than previously thought (where the Dsx proteins simply repress the genital primordium of the inappropriate sex). It is now clear that, depending on the genetic sex, Dsx proteins regulate the ability of cells to express and to respond to secreted signals. This role is reminiscent of the function of homeotic genes during development, as best illustrated by the homeobox transcription factor Ultrabithorax (Ubx). In wild-type, Ubx is expressed in the haltere, where it acts as a selector gene to repress the wing fate and direct the fate of the appendage from wing to haltere. During this developmental process, Ubx negatively regulates the activities of the two organizers of wing patterning, Dpp, localized along the A/P border, and Wg, expressed along the dorso-ventral border (Weatherbee et al., 1998). Thus, the functions of Dsx and Ubx in patterning of specific imaginal discs appear similar in that both are able to regulate the ability of cells to express and to respond to secreted signals.

Finally, genetic studies have revealed a cooperative interaction between *dsx* and another homeotic gene, *AbdB*. Since *AbdB* mutant clones in the genital discs revert to leg fate, AbdB specifies genital development in this disc in part by repressing leg development. AbdB antagonizes leg development by partially repressing the Wg and Dpp targets *dll* and *dac* (Estrada and Sanchez-Herrero, 2001). Thus, Dsx appears to be a regulatory partner of homeotic gene function by directing the "repressed leg" cells to become male or female genitalia. Future studies will be needed to precisely define how the information provided by Dsx cooperates with the position-specific information provided by AbdB, as well as the transcription factors that respond to both the Dpp- and Wg-secreted signals. As illustrated by studies in the wing (Guss et al., 2001), the identification of one or more genital-specific enhancers should help elucidate how these various regulatory inputs are integrated at the molecular level.

Conclusion

One of the central problems of developmental biology is how information from different regulatory hierarchies is integrated to regulate specific sets of genes in particular cell types. The genital disc provides a case where this integration occurs not at the promoters of the terminal differentiation genes, but instead at the level of the production and interpretation of extracellular signals. This finding illustrates a novel mechanism by which the limited number of conserved signaling networks and transcription factors can be deployed to direct differentiation of the organisms tissues. Future work will reveal how widely this developmental strategy has been used.

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