Tracing How the Sexes Develop

This Special News Report focuses on the rapid progress being made in unraveling the molecular biology of sexual development in organisms ranging from the fruit fly to humans

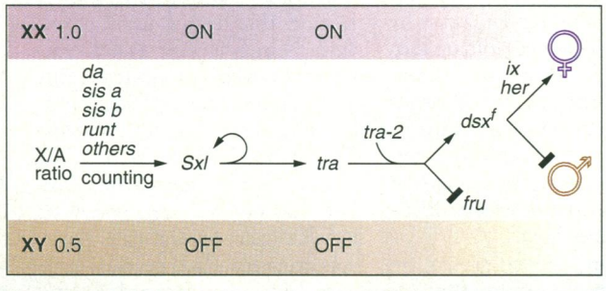
The contrasts between the sexes have inspired countless plays, novels, and other creative works. Sex differences inspire a group of developmental biologists, too—but there's a twist. While artists and most of the rest of us are fascinated by the effects of the male-female divide, these biologists are trying to learn how it arises in the first place. Their goal: to trace out the gene pathways that turn an embryo into a male or female. This quest has recently become one of the hottest areas of developmental biology, as two meetings held this year and devoted solely to the subject attest.*

In two organisms—the fruit fly *Drosophila melanogaster* and the simple nematode *Caenorhabditis elegans*—researchers are now close to realizing this ambition, having followed those pathways from their beginning at the sex chromosomes almost all the way through to their end. "If you think about the genes just purely devoted to this process, all or nearly all" have been identified in *Drosophila*, says Bruce Baker of Stanford University, who has been working on the fruit fly system for more than 20 years.

And developmental biologists are making great strides in deciphering the genetics of sex determination in mammals as well. They have identified a half-dozen genes whose involvement in sex determination is helping explain how the process sometimes goes awry in humans, causing genetic males to become partially or completely feminized and genetic females to develop male characteristics (see p. 1824).

The scientists engaged in these efforts aren't just satisfying their curiosity about how males and females, genetically so close, end up so different in anatomy and behavior. They are also exploiting sex determination as a model for understanding development generally. "It's a clean system to work with, because there's a choice between two distinct developmental fates," says one of the field's pioneers, Jonathan Hodgkin of the Molecular Research Council's Laboratory of Molecular Biology in Cambridge, U.K.

At the same time, sex determination is turning out to be a case study of how, in different organisms, evolution has resulted in dramatically different mechanisms for achieving the same end. When something is as fundamental as sex determination, says developmental and evolutionary biologist Nipam Patel of the University of Chicago, "it's easy for us to believe" that it's going to be similar in different species, as the genes that control other aspects of development tend to be. But the sex-determination genes in the fruit fly and the nematode are completely unrelated to each other, let alone to those in mammals. "What's mind-boggling is how few real similarities there are. All the players are different," says Thomas Cline of the University of California (UC), Berkeley. And that, says Patel, makes sex determination "a fascinating topic in terms of evolution."

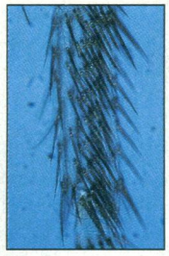


How flies do it. In females, activation of *Sxl* leads to production of a feminizing Dsx variant. Males, without active *Sxl*, make a different variant. Flies overexpressing the male *Dsx* have male sex combs along the entire leg *(right)*. A normal female leg is at far right.

In the beginning

In spite of the many differences between sex determination in *Drosophila* and *C. elegans*, the two species do have one thing in common: the nature of the primary signal that determines whether an embryo becomes male or female. Whereas the determining factor for mammals is the presence or absence of a Y (male) sex chromosome, what's important in the fruit fly and worm is the ratio of X chromosomes to sets of nonsex chromosomes, or autosomes, as they are called. In females, this X/A ratio is normally 1.0, as they have two X chromosomes and two copies of each autosome, while for males, with a single X, the ratio is 0.5. "The first thing the fly does is to count its X chromosomes," is how Cline puts it.

In the fruit fly, this counting is performed by a gene called *Sex-lethal* (*Sxl*), which acts as the master switch in sex determination. This role wasn't appreciated when the gene was first discovered by Hermann Muller in 1960, however. He called it *Female-lethal* because mutations that inactivate the gene kill female embryos, while males develop normally. Not until 2 decades later did Cline show that the gene is needed for sex determination. He found, for example, that the dying females were attempting to develop as males. By itself, this would not be fatal, but work by Cline's team and that of John Lucchesi of Atlanta's Emory University, among others, showed that the gene, renamed *Sex-lethal*, has a second, even more vital, role—in "dosage compensation," a process which doubles the activity of the genes on the male's single X chromosome to bring it up to that of the genes on the female's two.



PHOTOS BY VICKY JURSNICH AND KEN BURTIS

While this may sound trivial, it's not. In female flies, the Sxl protein is supposed to damp down the activity of the genes that increase X chromosome expression in males. That's why mutations that inactivate *Sxl* kill female embryos: They get a lethal overdose of the products of their own X chromosome genes. Dosage compensation is equally vital in mammals and *C. elegans*, although as with just about everything else in sex determination, these species rely on different mechanisms to accomplish it. (For details, see p. 1826.) "It's ironic that a twofold difference in gene expression makes a difference, but it's a matter of life or death," says Barbara Meyer of Berkeley, who studies sex determination and dosage compensation in *C. elegans*.

A twofold difference in X-chromosome gene expression is also crucial to *Sxl*'s chromosome-counting function. Before dosage

* "The Molecular Basis for the Difference Between the Sexes," sponsored by the Keystone Foundation and held from 12 to 18 February in Tamarron, Colorado; and "Mechanisms in Vertebrate Sex Determination," held on 24 and 25 May in London under the aegis of the Royal Society.

compensation kicks in, the gene essentially acts as a sensor for the difference in the expression of a small number of X chromosome genes. Work by several teams, including Cline's, has identified eight such genes. They encode transcription factors that when present in sufficient quantities in the early embryo activate *Sxl*, causing it to produce a special form of the Sxl protein. But that happens only in females, as only they have a double dose of the X-chromosome genes.

The corresponding master switch in *C. elegans* is *xol-1*, which Meyer and her colleagues have been studying for several years. Identification of the X-chromosome genes counted by *xol-1* is not as far along as the comparable work in the fruit fly, but work from Meyer's and Hodgkin's teams suggests that only a small number of genes is counted in this species, too. Hodgkin's team has reported identification of a possible candidate for one of these genes, and Meyer and her colleagues have pinned down at least three genes as *xol-1* regulators, including the one found by Hodgkin.

Entering the pathways

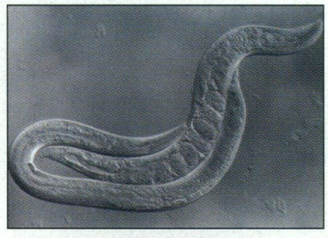
Once initiated, the sex-determination pathways in the worm and fly follow completely different routes. For one thing, the fruit fly master switch, *Sxl*, plays an active role only in female development. "*Sex-lethal* seems truly sex-specific, since you can delete it from males and they do just fine," Cline says. In contrast, the worm's master switch gene, *xol-1*, plays its critical role in masculinization, although it has a minor role in the female (actually a hermaphrodite because it produces a few sperm early in life in addition to eggs). Once the two genes are activated, other differences become apparent.

In the fruit fly, evolution has opted for a method of gene control known as alternative splicing. Like most genes in higher organisms, the genes in the fruit fly pathway contain noncoding sequences called introns, which have to be spliced out of the mRNAs copied from the genes before the mRNAs can be translated into proteins. And like many mRNAs, the ones in the fruit fly can be spliced in more than one way, affecting the activity of the resulting protein.

Cline's group, working with Paul Schedl's at Princeton University, has shown that the Sxl protein made when the gene is first switched on in female embryos is a splicing regulator. Its targets are transcripts made from the same gene, which later in development becomes active for a second time in females and for the first time in males. The initial Sxl protein controls the splicing of this second RNA in females so that it produces a functional protein, while the male transcript makes an inactive protein.

And the alternate splicing theme continues throughout the fruit fly sex-determination pathway. The active Sxl also works on the mRNA made by *tra* (*transformer*), the next gene in the pathway, to bring about formation of still another female-specific splicing regulator. Its target is the final gene in the pathway, called *doublesex* (*dsx*), which Baker's team showed is also regulated by alternative splicing.

Acting in concert with the protein made by another gene, *tra-2*, the Tra splicing regulator results in the expression of one variant of the Dsx protein in females. In males, which do not have a functional Tra protein, a different Dsx protein is produced. Either way, the product is a transcription factor, but the results are dramatically different as male versus female. As Baker says, "*Doublesex* shows how you can have a bifunctional gene that makes two active [protein] forms."



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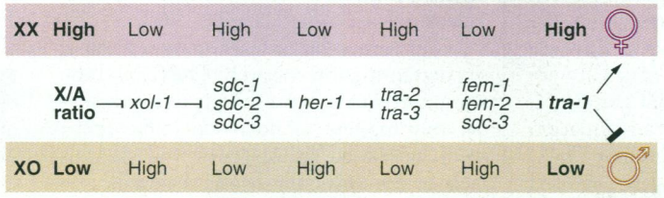


The worm's way. In *C. elegans*, it's the male who needs active gene input, in this case from *xol-1*. In the mating pair of worms above, the hermaphrodite is shorter and fatter than normal because of a mutation.

The exact ways in which the genes in the *C. elegans* sex-determination pathway interact have not yet been fully worked out, but alternate splicing does not appear to play the dominant role that it does in the fly. Instead, the proteins encoded by the worm genes may set up a system for transmitting sex-determining signals between two or more communicating cells. That's a major difference from *Drosophila*, where, for most somatic cells, the entire series of gene changes takes place within individual cells.

As Meyer and her colleagues showed earlier this year, sex determination in the worm gets under way when *xol-1* expression goes up in response to the male X-to-autosome ratio of 0.5. High *xol-1* activity in turn leads to reduced activity of the next genes in the pathway, which are called *sdc-1*, *-2*, and *-3* (where *sdc* stands for sex and dosage compensation). Because the proteins produced by these genes reduce expression of *her-1*, the next gene in the pathway, inhibition of the *sdc* activity in males causes production of the Her-1 protein to increase.

And Her-1 appears to be designed to carry signals between cells, because the *her-1* sequence, which was determined by Bill Wood's team at the University of Colorado, Boulder, indicates that the gene encodes a secreted protein. What's more, the next gene in the worm pathway, *tra-2* (not related to the fly's *tra-2*), which was cloned and sequenced by Judith Kimble, Patty Kuwabara, and their colleagues at the University of Wisconsin, Madison, likely makes a membrane-bound receptor protein.

Taken together, these results suggest that at least two different cell types interact with each other during *C. elegans* sex determination, with the signal between them being transmitted by Her-1 acting through the receptor protein Tra-2. "The cells are talking to each other. The message they are sending is that I'm masculine and I want you to be masculine, too," says Marc Perry, who worked on the system in Wood's lab and is now at the University of Toronto.

Following the trend in the *C. elegans* pathway, the Her-1 signal is itself inhibitory, reducing Tra-2 activity and leading in turn to increased activity of the next genes, *fem-1*, *-2*, and *-3*, in males. And finally, the Fem proteins tell the nucleus to makes less of the protein product of the last gene in the pathway, *tra-1*. *Tra-1* encodes a transcription factor, which Hodgkin's group has shown triggers female development. The reduction in its activity in males thus masculinizes the embryo.

End game

There may still be a few details missing from this picture. At the sex-determination meeting held in Tamarron, for example, Betsy Goodwin of Northwestern University School of Medicine in Chicago presented as yet unpublished results suggesting that there may be another, feminizing gene in *C. elegans* in addition to *tra-1*. But researchers now have a clear view of most of the genes controlling primary sex determination in both the fly and nematode.

What happens next to bring about the development of the sex organs and related structures is still largely a mystery, however. Both *dsx* and *tra-1*—the last known genes in the two pathways—encode transcription factors, but for the most part their targets are unknown. "In all of the systems, whether it be flies or worms or mammals, we have a lack of downstream genes. It's been hard to find them, a much tougher problem than people expected," says Hodgkin.

One problem is that the Dsx and Tra-1 transcription factors bind to short DNA sequences, containing only six to nine base pairs. Because such short sequences can occur randomly hundreds or even thousands of

times in the genome, they can't readily be used to pick out target genes.

Another is that a key source of clues to the primary sex-determining genes—mutations that cause well-defined abnormalities in sexual development—hasn't been very fruitful for the downstream target genes, says Ken Burtis of UC Davis. That may be, he explains, because the genes needed to build the sex organs are also needed to make other organs and tissues. Many of these genes may therefore be necessary for life. As a result, "you may get a dead embryo with an uninformative phenotype," Burtis says.

Even so, one set of target genes has been tracked down: Work by Burtis, Baker, and Pieter Wensink at Brandeis University in Waltham, Massachusetts, has shown that the genes encoding *Drosophila* yolk proteins, expressed only in female fat bodies and ovaries, are direct regulatory targets of the Dsx proteins. In addition, Hodgkin's group and that of Scott Emmons at Albert Einstein College of Medicine in New York City are investigating the possibility that certain *C. elegans* genes involved in male tail formation and in egg-laying may be regulated by Tra-1.

Besides finding the target genes needed for development of the sex organs, researchers would also like to find out what happens in the brain to bring about the characteristic sexual behaviors of males and females. As Barbara Taylor of Oregon State University said at Tamarron, "The nervous system is pretty much of a black box" in this regard, except for hints of the influence of a *Drosophila* gene called *fruitless* (*fru*), which Jeff Hall's group at Brandeis has implicated in male mating behavior. Males with *fru* mutations court both males and females indiscriminately, and are sterile because they don't complete the mating act for reasons that are not yet understood. Recent work by Taylor, Lisa Ryner of Stanford, Baker, and Hall indicates that the *fru* gene functions in the sex determination pathway of the fly at a branch after *tra* and *tra-2*, with *dsx* and *fru* lying on the separate branches.

Researchers are also taking up the challenge of working out how evolution produced such a panoply of mechanisms for sex determination. This work is still in its early stages, but as Patel points out, "Clearly you're at the point now where you can approach [sex evolution]." As these and the other studies unfold, developmental biologists might well like to join the proverbial Frenchman in exclaiming, "Vive la différence."

–Jean Marx

Additional Reading

S. M. Parkhurst and P. Meneely, "Sex Determination and Dosage Compensation: Lessons from Flies and Worms," *Science* **264**, 924 (1994).

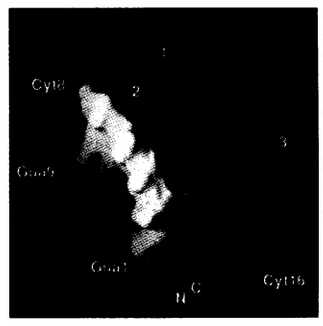
L. S. Ryner and A. Swain, "Sex in the '90s," *Cell* **81**, 483 (1995).

MAMMALIAN SEX DETERMINATION

Snaring the Genes That Divide The Sexes for Mammals

When it comes to sex, flies and worms are way ahead of mammals, at least in the eyes of molecular biologists. Developmental biologists have been able to work out the detailed genetic pathways that control whether an embryo of the fruit fly or the nematode worm develops as male or female (see p. 1822). Mammals are much more difficult to study, but within the past 5 years, developmental biologists have uncovered a half-dozen genes that play a role in mammalian sex determination, and are beginning to trace out some of the early biochemical pathways that divide the sexes.

These genes illustrate a common trend in sex determination research. The fly and the worm turn out to have sharply different sex-determining genes. And mammals have yet a third, unrelated set of genes. Indeed, this lack of evolutionary conservation is one factor that has made mammalian studies difficult, because researchers can't use information gleaned from the simpler creatures to guide them in their work. "In some ways, it's been frustrating," notes Robin Lovell-Badge of the National Institute for Medical Research in Mill Hill, U.K., one of the pioneers of the mammalian work. But he quickly adds that the fact that the mammalian genes are totally different "is also more interesting, because it means we are looking at something new."



M. H. WERNER *ET AL.*, *CELL* **81**, 705 (1995).

Bent. DNA bends sharply on binding a segment of the human SRY protein *(green)*.

By looking at these new genes, researchers are learning about more than normal mammalian development. In several cases, the key clues that enabled researchers to identify mammalian sex-determination genes came from studies of people with disorders of sexual development, in which individuals who should belong to one sex genetically end up with some or all of the reproductive organs of the other. Part of the work's appeal is the light it can shed on these disorders—and on other developmental abnormalities, because some of the sex-determining genes in mammals also influence the development of other organs. Studies of sex-reversed humans, in fact, helped researchers home in on the "master switch" for mammalian sexual development: a gene called *SRY*.

One of the first clues to *SRY*'s existence came 40 years ago in experiments by the late Alfred Jost. Jost surgically removed the gonads of embryonic rabbits in utero before the other sex organs formed and found that male embryos failed to develop internal and external reproductive organs, while females developed normally. This implied that the embryonic testis is all that is needed to bring about development of the other male sex organs. And that, of course, raised the question of what causes the testis to develop.

A big clue came a few years later when researchers showed that the gene for the testis-determining factor (TDF), as it was called, must reside on the Y chromosome. They found, for example, that either humans or mice having a single X sex chromosome—instead of the normal two X chromosomes of females or the X plus Y chromosome of males—develop as females. In contrast, individuals who have a Y chromosome develop as males, even if they have two or more X's.

Another 30 years would pass before the gene was actually found, but the pace began to pick up in the mid-1980s. By mapping the chromosomal abnormalities linked with sex reversal in humans—loss of part of the Y chromosome in XY females, for example, or gain of a Y chromosome segment in XX males—researchers pinned down the location of the TDF gene to approximately 35 kilobases of DNA on the short arm of the Y chromosome.

In 1990, teams led by Lovell-Badge and by Peter Goodfellow of the University of Cambridge, U.K., isolated the TDF gene, which they named *SRY* (for sex-determining region, Y chromosome), using positional cloning. "We knew there was a gene on the Y chromosome responsible for the decision to make a testis," Lovell-Badge says, and there is "sufficient proof" that it is *SRY*.

Part of that proof came from studies of mice: The researchers found that the gene becomes active in the developing gonads just before the tissue begins specializing to form a testis. What's more, when the Lovell-Badge–Goodfellow team introduced the gene into newly fertilized mouse eggs, it caused genetic females to develop into males.

Further studies of sex-reversed individuals show that *SRY* is also needed for testis formation in humans. Researchers, including Goodfellow's team and that of Ken