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).

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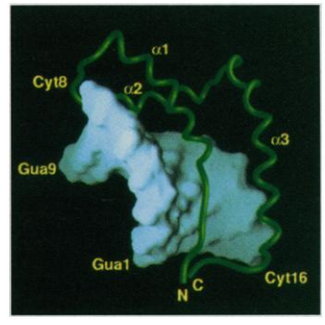
MAMMALIAN SEX DETERMINATION

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

McElreavy at the Pasteur Institute in Paris, have found that *SRY* is mutated in about 25% of XY females. And conversely, examination of the small Y segments found in XX males confirms that the gene is present there. Indeed, says Keith Parker of Duke University Medical School, "There's compelling genetic evidence that *SRY* is the linchpin for the whole thing [testis determination]."

Exactly how *SRY* brings about testis formation is unclear, although its sequence provides an important clue. It shows that the SRY protein carries a DNA binding sequence, known as the HMG box, and may therefore be a transcription factor that alters the expression of other genes by binding to their DNA. Several lines of evidence support that view.

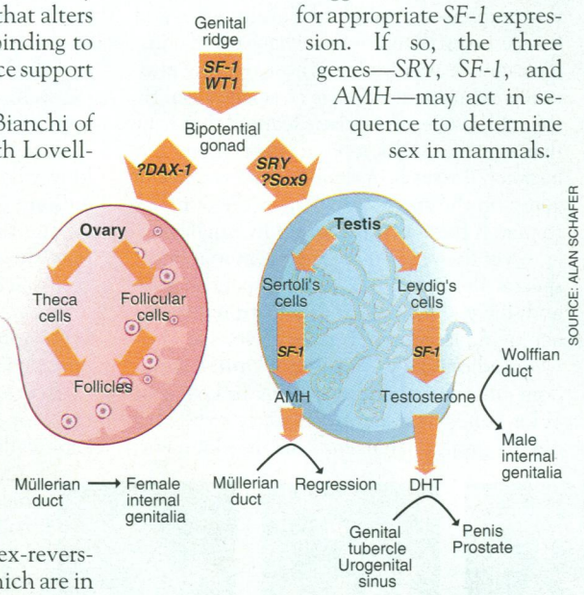
Last year, for example, Marco Bianchi of the University of Milan, Italy, with Lovell-Badge, Goodfellow, and their colleagues, provided indirect evidence that SRY binding causes DNA to bend sharply through an angle of 70 to 80 degrees. And earlier this year, Marius Clore's group at the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, Maryland, confirmed that by determining the three-dimensional structure of a complex between the HMG domain of human SRY and DNA.

Bianchi went on to show that sex-reversing *SRY* mutations, almost all of which are in the HMG box, either reduce SRY binding to DNA or the bending it causes. These results suggest that normal SRY changes the architecture of the DNA, thus allowing access of other factors needed for gene expression. The mutations apparently prevent that. Lovell-Badge notes, however, that while "we know in some ways how [SRY is] working, the biggest question is what are the downstream targets."

The best candidate so far is the gene encoding a protein called anti-Müllerian Hormone (AMH), which is produced by the testis soon after it develops. *AMH*'s job is to cause the breakdown in males of the embryonic structure called the Müllerian duct, which gives rise to the internal female organs including the oviducts and uterus.

Because AMH production comes on after SRY, it could be a target of SRY regulation—but it may be an indirect one. Recent work from Duke's Parker and Holly Ingraham of the University of California, San Francisco, and their colleagues suggests that another transcription factor might be the immediate *AMH* gene regulator. Parker's team discovered the protein, called steroidogenic factor 1 (SF-1), about 4 years ago as a global regulator of the genes for all the enzymes that make steroids. But when the Duke team knocked the *SF-1* gene out in mice, they got a surprise. "In addition to the enzymes, the animals lost their kidneys and their gonads," Parker says. "[*SF-1*] had to be doing more than making steroids."

Ingraham and her colleagues then provided an idea of what that something more might be. They showed that the *AMH* gene's regulatory sequence contains a binding site for SF-1 and that this site is necessary for the gene's activity, although they have not yet been able to show that SF-1 binding does in fact turn on the gene. The Ingraham-Parker team's results also suggest that *SRY* is needed for appropriate *SF-1* expression. If so, the three genes—*SRY*, *SF-1*, and *AMH*—may act in sequence to determine sex in mammals.



SOURCE: ALAN SCHAFER

Following the trail. The diagram shows the possible functions of the genes linked thus far to mammalian sex determination: *SRY*, *Sox9*, *AMH*, *WT1*, *SF-1*, and *DAX-1*.

At least two other genes may also influence mammalian sex determination. In both cases, the clues have come primarily from studies of rare human genetic disorders whose symptoms include abnormal sex organ development. One of these is Denys-Drash syndrome (DDS). Patients with this condition usually die of kidney failure by age 2, but the genetic males also have partly feminized reproductive systems.

About 3 years ago, two research teams, one led by David Housman of the Massachusetts Institute of Technology and the other by J. Cowell of the Imperial Cancer Research Fund in London, found that DDS is caused by mutations in the Wilms tumor (*WT1*) gene. Further evidence that *WT1* is needed for normal sexual development came when the Housman team, with that of Rudolf Jaenisch of the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, knocked out the gene in mice. As a result, the animals lacked both kidneys and gonads, as well as having other abnormalities.

Exactly how *WT1* relates to the other mammalian sex-determination genes is unclear, but Jerry Pelletier of McGill University in Montreal, who contributed to both the DDS and mouse studies, says that its expression comes on in the developing gonad before that of *SRY*. Because *WT1* encodes a transcription factor, it might be involved in turning on *SRY*. But even though Pelletier expects that *WT1* regulates something, he cautions that as yet, "we just don't know what."

The second hereditary disease that has pinpointed a gene involved in mammalian sex development is called campomelic dysplasia. The major symptom of this disease is severe skeletal abnormalities, which lead to death shortly after birth, but about two thirds of genetic males who have it also have completely or partially feminized sex organs. Last year, two groups, one led by Goodfellow and Cambridge colleague Alan Schafer and the other by Gerd Scherer of the University of Freiburg, Germany, found that campomelic dysplasia is caused by mutations in a gene called *Sox9*. "This particular gene seems to be a component of the pathway for mammalian sex determination," Schafer says. Where it fits into the pathway is unclear, however, although *Sox9*, like *SRY*, carries an HMG box and may therefore be a transcription factor.

The list of mammalian sex determination genes is not likely to stop there. As McElreavy points out, "Mutations in *SRY* can explain only a small proportion [about 25%] of XY sex reversals." Indeed, a strong candidate that may explain some of the other cases is already on the horizon: a gene called *DAX-1* discovered late last year by a multinational team led by Giovanna Camerino of the University of Pavia, Italy. Mutations in *DAX-1* cause X-linked adrenal hypoplasia congenita, a developmental disorder in which the adrenal gland fails to develop normally.

The reason for thinking that *DAX-1* is important for mammalian sex determination is that it maps to a small region of the X chromosome that the Camerino team had previously found to be duplicated in XY females. This lead them to suggest that the region carries a gene needed for development of the ovaries, a double dose of which might feminize the developing gonad of males. *DAX-1*'s location makes it a candidate to be that gene, although that has not yet been proven.

If it can be, *DAX-1* would be a significant new addition to a diverse collection of mammalian sex determination genes. And researchers are confident that the collection will expand as they dig deeper into the mysteries of sex determination. Indeed, it may not be long before mammals finally match the sex appeal of worms and flies.

–Jean Marx

Additional Reading

A. J. Schafer, "Sex Determination and Its Pathology," *Advances in Genetics* **33**, 275 (1995).