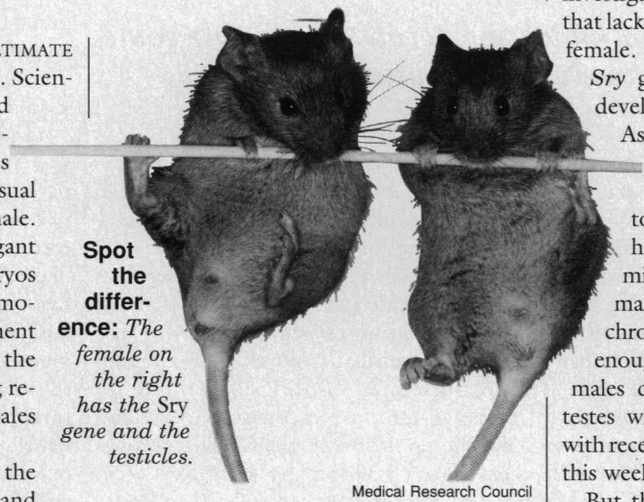
# Sex and the Single Gene

## *British scientists find that all it takes to make a man is a tiny fragment of DNA*

*London*—THE HUNT FOR THE ULTIMATE genetic source of masculinity is over. Scientists in Britain confidently announced yesterday that they had ended the 30-year search for the gene that switches mammalian development from its usual destination—the female—to the male. The proof comes in a simple but elegant experiment: When female mice embryos carrying the normal pair of X chromosomes are injected with a small fragment of Y chromosome DNA containing the *Sry*-gene (short for sex-determining region Y gene), they grow up as males with testes and male behavior.



**Spot the difference:** *The female on the right has the* Sry *gene and the testicles.*

Medical Research Council

The experiment ends a race for the gene that has shuttled between mice and men and stumbled down several blind alleys. Three years ago it looked as though the search might be over when David Page and colleagues at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, announced in *Cell* that they had "probably" found "the master sex-determining locus." But that result proved to be a near miss, and the prize now goes to a British team, led jointly by Robin Lovell-Badge of the National Institute for Medical Research and Peter Goodfellow of the Imperial Cancer Research Fund, who are publishing their results in this week's *Nature*.

What Lovell-Badge and his colleagues have identified is the genetic switch on the Y-chromosome that triggers the genital ridges in the embryo to develop into testes rather than ovaries. Once that has happened, all the other changes that make a male animal follow under the influence of hormones produced by the testes.

Scientists have known since early in the century that males have different chromosomes than females. In mammals, males have an X and a Y chromosome and females have two X chromosomes. But it wasn't until 1959 that the presence of the Y chromosome was shown to be essential for the development of males in mice and humans.

Since then, the hunt for the gene that switches development to the male pathway has closed in on ever-smaller regions of the Y chromosome. An early candidate was the so-called H-Y gene, which coded for an immune-system protein found only in males, but in 1984 Anne McLaren, an embryologist at the Medical Research Council's Mammalian Development Unit in London, discovered mutant mice that completely lacked the H-Y protein but were "indisputably male" and developed testes.

Attention then shifted from mice to men. Clues came from two groups: XX men who were male because they had inherited a small fragment of the Y chromosome from their fathers, and XY females who had lost a crucial part of their Y chromosome. It was one of these females that led David Page to his close encounter with the testes-determining factor gene (labeled *TDF* in humans and *Tdy* in mice). The woman was missing a very small region from her Y chromosome, which Page and his group were able to clone and sequence. They found that the missing 140 kb segment contained just one gene, whose sequence suggested it coded for a protein with many "fingers"—loops of amino acids formed by zinc cation bridges—that would be ideal for binding DNA and thus regulating gene activity.

But while Page's "zinc-finger" protein, ZFY, was the closest anyone had come to *TDF*, further work was soon to show that it was not the gene that determined sex.

In London, two strands of evidence absolved *ZFY*. "[Peter] Goodfellow at the Imperial Cancer Research Fund found DNA from XX males with no *ZFY* genes," explains Peter Koopman, one of Lovell-Badge's team, "and we showed that in the mouse *Zfy* was active at the right time but not in the right cell type."

With the prize still unclaimed, Goodfellow joined forces with Lovell-Badge, and within 6 months they made their big breakthrough. The British team found that human XX males who lacked *ZFY* had inherited another small piece of Y chromosome, lying close by. Together, Goodfellow and Lovell-Badge established quickly that this 35 kb region contained a single gene, which they called *SRY* for sex-determining region Y. Male mice contain a very similar region, *Sry*, as do many other mammals. Further investigation turned up a strain of XY mice that lacked the *Sry* gene andwas biologically female. And experiments showed that the *Sry* gene was active only as the testes develop in the embryo.

As promising as these findings were, Goodfellow and Lovell-Badge were not willing to declare victory until they had final proof. That had to await the birth of transgenic mice, which contained the normal female XX chromosomes plus 14 kb of Y chromosome bearing the *Sry* gene. Sure enough, some of these chromosomal females developed into males with normal testes who behaved normally when caged with receptive females. Hence the assertion in this week's *Nature* that "*Sry* is *Tdy*."

But the story isn't completely over yet. "Not all of the transgenic females became boys," McLaren told *Science*. "Why not?" Lovell-Badge's group is looking to see whether *Sry* is expressed in those females that stayed female. "We don't know what to expect," said Koopman. "If they're not expressing, that's clear. If they are, it may be a question of the timing or level of activity."

Lovell-Badge stresses that *SRY* is only a switch. Testes may develop in XX women who have no portion of the Y chromosome, presumably because mutations elsewhere allow them to do so, and in cattle some genetic females become freemartins—cows with testes—in response to hormone blockers. That shows that the genes needed to turn the "indifferent" genital ridge of the embryo into male gonads are present in the female. But the gene that normally triggers this change—*SRY*—is present only in the male.

And, of course, pinning testes determination on *Sry* is the end of one quest but the beginning of another: Something controls *Sry*, and *Sry* controls other genes. "I think they're going to start a great hunt for the rest of the genes in the cascade," said McLaren. In fact, McLaren is particularly interested in "XY women who have no mutation in their *SRY* gene as far as we know." Why don't they develop testes? Could there yet be a different mutation "upstream" of *SRY*?

"The next steps are the difficult ones," said Lovell-Badge: "To identify the genes with which *SRY* interacts, those that turn it on and off in such a dramatically short period, and those that it in turn regulates." McLaren puts it more succinctly: "In the end we may actually understand how sex is determined in mammals." ■ JEREMY CHERFAS