CHOOSING A SEX

THERE IS MORE TO BECOMING A MAN THAN HAVING A Y CHROMOSOME. RARE SEX REVERSAL SYNDROMES HAVE SHOWN THAT A CASCADE OF GENES IS NEEDED BOTH TO PROMOTE MALE DEVELOPMENT AND TO COUNTERACT SOME SPIRITED OPPOSITION FROM FEMALE-FAVOURING GENES.

TEXT WILLIAM WELLS

Charlie Chase was born in a suburb of New York in 1956. He had ten fingers; he had ten toes. But something was very wrong. His testes had not descended, and he possessed something halfway between a small penis and a large clitoris. "The doctors completely freaked out and couldn't deal with my parents," says Chase. "They kept my mother sedated for three days until they decided what to do."

Based on the size of his genitalia, Charlie was to be raised as a boy. But 18 months later a second medical team discovered that Charlie had mixed ovarian and testicular tissue – defining him as a true hermaphrodite – as well as a vagina and uterus. Fertility was seen as the primary goal, so Charlie's penis/clitoris was cut down to the 'normal' size for a clitoris, and Charlie was renamed Cheryl.

Through another surgery at the age of 8 and a generally miserable childhood, Cheryl was kept in the dark about her condition. In her twenties, after three years of campaigning to find out the truth, she was finally handed three pages of her medical notes and sent on her way. "I was suicidal and filled with rage, but also crippled with shame," she says. "Surgery didn't protect me from that; in fact, it probably caused it."

Cheryl Chase founded and is today the director of the Intersex Society of North America (ISNA). The society's mission is to demystify intersex conditions and delay cosmetic surgery until the child can voice his or her opinion. Although true hermaphroditism is very rare, intersex conditions are not, with perhaps five intersex babies born every day in the United States.

The vast majority of intersex conditions are the result of blocked, ill-timed, or mixed hormone messages from what are initially normal gonads (testes or ovaries). Although the ultimate cause of Cheryl's condition is unknown, it is more likely to have been brought about by a failure in the much earlier process of sex determination, when the gonads themselves are first produced.

GENES AND GENDER

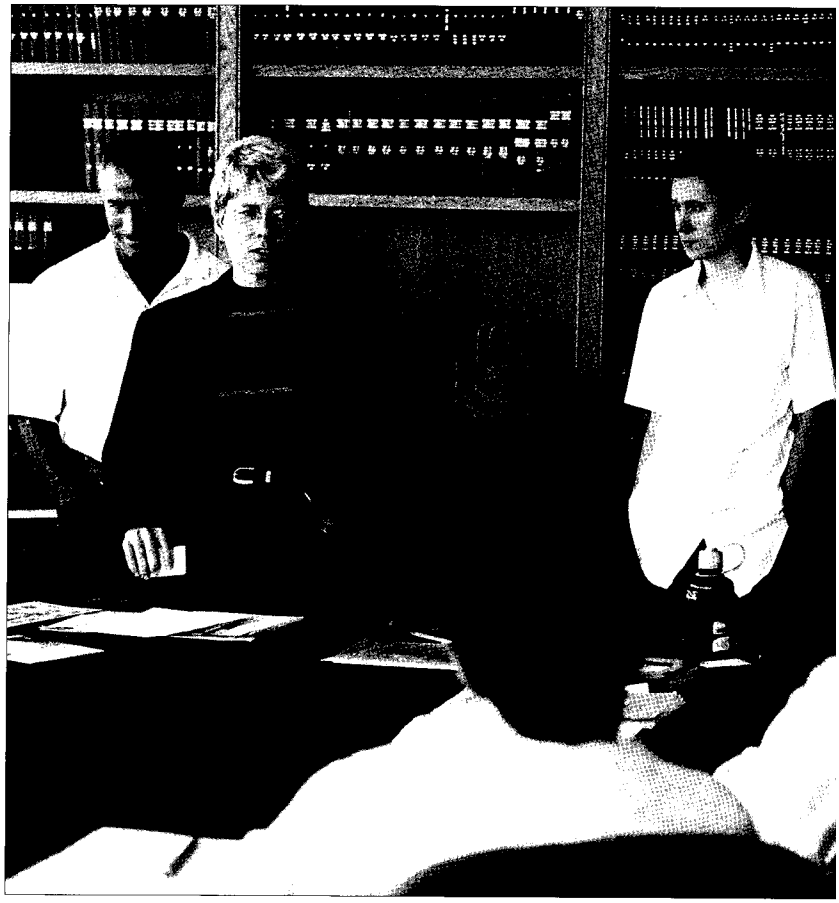
The 'normal' male carries one X chromosome and one Y chromosome (XY) and the 'normal' female carries two X chromosomes (XX). Aberrant sex determination during early development most often results in individuals who appear nearly normal, but whose sex defies the underlying sex chromosomes, *i.e.* XX males or XY females. By studying these rare cases, researchers are beginning to build up a picture of how a piece of tissue, initially identical in the male and female embryo, can be triggered to develop into one of two identities: a testis or ovary. "The gonad really is bipotential, but the system is such that, once the switch is thrown, then all the cells in the gonad join the plan," says cell biologist Blanche Capel, at Duke University in Durham, North Carolina. "It's a brilliant system for looking at how organs are built, because there is no other organ that is bipotential in this way."

The Y chromosome is the starting point for the genetic process of sex determination, in as much as having a Y chromosome generally makes you a man. Although, →

theoretically at least, the number of X chromosomes could just as easily be the important determinant (as occurs in species such as the worm), our experience with chromosome abnormalities has confirmed that in humans sex determination depends on the Y chromosome. For example, individuals with Turner syndrome carry just one X chromosome and no Y chromosome (*i.e.* they are X0), and still appear female. Individuals with Klinefelter syndrome have two X chromosomes as well as a Y chromosome (*i.e.* XXY), yet still appear male.

The realization that the Y chromosome co-ordinates sex determination triggered the search for a critical gene on the Y chromosome – a putative 'testis-determining factor' gene. The theory was that if a single, critical testis-determining factor gene does exist then all males should have the gene and all females should not, whatever complement of X and Y chromosomes, or indeed portions of X and Y chromosomes, they carry.

In an attempt to home in on the critical gene, David Page at the Massachusetts Institute of Technology in Cambridge, Massachusetts, focused on a stretch of the Y chromosome that harboured three important gene-carrying regions. In 1987 he reported that genetic analysis of two intersex patients apparently confirmed that one of these regions carried the testis-determining factor gene. A quick search highlighted a candidate gene known as *ZFY* (the Y-chromosome-specific zinc finger gene).

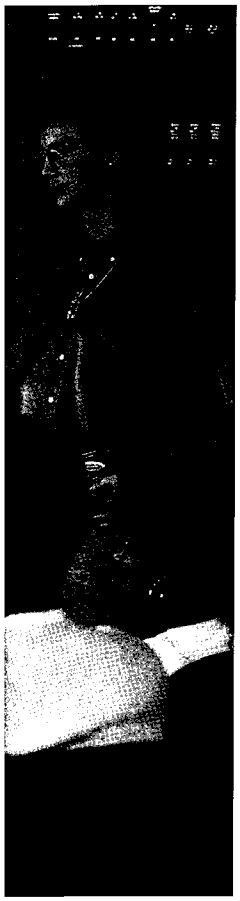


The *ZFY* gene makes a protein, ZFY, that resembles a well-characterized group of proteins known as transcription factors. Transcription factors act to control the production of other proteins, and this was just the sort of activity expected of a candidate testis-determining factor. "Most people believed it," says Robin Lovell-Badge at the National Institute of Medical Research, London, UK. "I was one of the few that didn't quite trust it. There were a number of inconsistencies, any one of which could be explained away, but together they made me suspicious."

Cracks did indeed begin to appear in Page's hypothesis that *ZFY* was the testis-determining factor gene on the Y chromosome. Unlike in humans, *ZFY* was found on the non-sex chromosomes in marsupials, and it was missing in some XX 'men'. Lovell-Badge and colleague Peter Goodfellow started a search within another region of the Y chromosome, and in 1990 identified another promising transcription factor gene, called *SRY*, or 'Sex determining Region Y'. Apparent confirmation that *SRY* was indeed the postulated testis-determining factor gene was made when XY females were found to carry mutations in *SRY*. The assumption was that these individuals developed into females despite being XY, because a mutation in the critical *SRY* gene meant the resulting SRY protein could not function properly to ensure the development of male gonads. The clincher in favour of *SRY* came when Goodfellow and Lovell-Badge managed to convert an otherwise normal XX (*i.e.* female) mouse to a male, simply by adding an *SRY* gene.

SRY was the subject of countless papers in the journal *Nature*, and its status as the testis-determining factor gene was never seriously challenged. But the story has since evolved considerably. Only about 15 per cent of XY females have been found to carry *SRY* gene mutations, and further genetic study of the remaining 85 per cent of these individuals has led to the discovery of additional sex-related genes.

Moreover, if *SRY* really is the critical genetic switch in sex determination, the gene should be almost identical (*i.e.* conserved) in different species. Comparing different mammals failed to confirm this; in fact the similarities between *SRY* in different species are barely recognizable. And while Lovell-Badge had predicted that the protein made by the *SRY* gene would act to control the activity of a host of other genes, further investigation showed that the bits of the protein that might fulfil such a task were either variable between species or missing altogether. "This was a very strange observation," says Jenny Graves, an evolutionary



Cheryl Chase (second left), founder and director of the Intersex Society of North America (ISNA) speaking at UC Berkeley in 1999.

Intersex is messy, there is a lot of variation in genital appearance even with identical genetics

biologist from La Trobe University in Melbourne, Australia. "*SRY* seemed like a very unusual and pathetic relic of a gene."

The only part of the SRY protein that looked at all similar between species was a short stretch that was shown to latch onto DNA. This observation ended up turning the original theory of *SRY*'s function on its head. Experts speculated that, rather than being a 'switcher on of pro-male genes', *SRY* may in fact be latching on to specific regions of DNA in order to obstruct the expression of a gene or genes that would otherwise promote feminization. The suggestion was surprising, because the female state is generally characterized not by the activation of feminizing genes, but by a lack of pro-male signals. If there is no Y chromosome, there is no *SRY*, no testosterone (and therefore no development of the Wolffian duct into the sperm-carrying vas deferens), and no Müllerian inhibiting substance (which in males turns off the Müllerian duct so it doesn't develop into a uterus and vagina).

The contrast to all these pro-male activities comes from the *DAX1* gene, (dosage-sensitive sex reversal-Adrenal hypoplasia congenita critical region on the X chromosome gene 1), which may be the gene with which SRY interferes. It is actively anti-male, and perhaps even pro-female, and must be inhibited by SRY for true maleness to develop.

"The theory in the field is that there is a war going on in the early gonad over which gene [*SRY* or *DAX1*] is going to gain control," says Capel. Studies in mice have even shown that if activation of the *SRY* gene to produce the SRY protein occurs too late, then *DAX1* triumphs. "That's an important conceptual advance," says Andy McMahon at Harvard University in Cambridge, Massachusetts. "Rather than considering a dominant male gene that always wins out, you have a more balanced scenario where the timing is critical. The realization over the past five or six years has been that there is a female pathway as well." It is now evident that the generation of either male or female gonads during embryonic development is almost certainly controlled by a number of interacting and in some cases competing pro-male and pro-female genes and proteins.

UNDERSTANDING INTERSEX

One or more of these genes might prove relevant to Cheryl Chase's medical history. One possible explanation for her hermaphroditism already exists. The explanation begins with a phenomenon called dosage compensation. All female (XX) cells naturally switch off one copy of each X-related gene so that they produce the same quantity of X chromosome-related proteins as male cells, which carry only one X chromosome. But not all female cells switch off genes on the same X chromosome; rather, different groups of cells can inactivate genes from either one of the X chromosomes. This is of no consequence if both X chromosomes carry identical genes. However, if – as occurs in about 10 per cent of XX hermaphrodites – one of the X chromosomes also carries an *SRY* gene (which of course is normally located on the Y chromosome), a cell that switches off the X chromosome carrying the *SRY* gene would become ovarian. If the other X chromosome is turned off, however, the active *SRY* gene would make the cell testicular. The resulting person would almost certainly display both testicular and ovarian cells.

Chase has given blood for an *SRY* test, but has yet to hear the results. Not that it particularly matters to her – a genetic explanation will not change who she is, or the lives of future hermaphrodites.

The test is not a clincher for Chase or others because the status of a single gene often fails to predict the predominant sex of a child. "Intersex is messy," says Chase. "There's a lot of variation in genital appearance even with identical genetics." For example, two siblings with identical portions of a Y chromosome have been successfully raised as an unambiguous male and an unambiguous female, and two XY sisters owe their gender identity to a defective *SRY* gene inherited from their apparently normal father. And if knowing the mutation doesn't predict the sex, it certainly doesn't solve any social problems. "You need a much more human kind of view to take care of intersex families," says Chase. "You need to listen to people." ■

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