Zeroing in on the Sex Switch

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Science, New Series, Vol. 239, No. 4835. (Jan. 1, 1988), pp. 21-23.

Stable URL:
http://links.jstor.org/sici?sici=0036-8075%2819880101%293%3A239%3A4835%3C21%3AZIOTSS%3E2.0.CO%3B2-Y

Science is currently published by American Association for the Advancement of Science.

Zeroing in on the Sex Switch

The discovery of a gene on the Y chromosome that apparently determines the sex of a developing embryo brings closer to resolution one of biology's most ancient problems

THE Greeks believed it was the level of male excitement during intercourse that determined whether the child would be male or female—the more excited the man, the greater the likelihood of siring a boy. David Page, of the Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, thinks it is a single gene, which he has recently pinpointed on the Y ("male") chromosome and cloned. Judging by its structure, the gene encodes a protein that controls the expression of other genes.

However, it appears that sex determination almost certainly is not a simple "on-off" effect, because Page and his colleagues have now discovered this same gene—or something very like it—on the X ("female") chromosome. Clearly, something quite subtle must be going on. "This may really shake up people's ideas about sex determination," said Page at the public announcement of the results at the end of December.

Since 1959 it has been known that in humans and other mammals the Y chromosome determines sex, unlike invertebrates in which the X chromosome is sex-determining. (Human embryos that inherit an X and a Y chromosome develop as males, those who inherit two X chromosomes, as females.) Thus, it was reasoned, a gene or genes must exist on the Y chromosome that act as a "master switch" to set in motion the cascade of events that lead to sexual differentiation.

For the past few years Page has been scouring the Y chromosome for this elusive gene, known as the testis-determining factor, or TDF, because its presence or absence determines whether the fetal gonad develops into testes or ovaries. In early development the human fetus is "indifferent"—that is, males and females are histologically indistinguishable. At about week 6 or 7, a switch is flipped and the primitive gonad develops into either testes or ovaries, the first and crucial step in the pathway of sex differentiation. With that decision, the die is cast: the differentiating gonad secretes hormones that govern the subsequent stages of sex differentiation.

Page, who last year won a McArthur Foundation "genius" award and who calls his lab at the Whitehead Institute the XY Corral, stumbled onto the search for the TDF gene in 1981, almost by accident, when he was gearing up to work on a genetic linkage map of the human genome. Maps are constructed from DNA probes, distinctive pieces of DNA that can be used as markers for genes. One of the first probes Page found had sequences common to both sex chromosomes—something that had not been detected before. Not only did the probe raise intriguing questions about how the two sex chromosomes evolved, it also provided a tool for studying them. Page abandoned the linkage map and joined the handful of laboratories looking for the TDF gene.

It was "sex-reversed" individuals, whose sexual identities are at odds with their chromosomal makeup, that led Page to the putative gene. These include XX males (males who have two X chromosomes instead of the usual XY makeup), XY females, and several other variations on the norm. "This is a classic use of very rare human genetic defects to find something very important about biology," says David Baltimore, director of the Whitehead Institute.

"The key to the whole endeavor rests with certain exceptions to the rule that Y is sex-determining," explains Page. "XX males were the most important exception." XX males appear entirely normal—their unique chromosomal makeup does not usually come to light until they try to have children and are found to be sterile. When Page began this research, no one knew how the testes could develop in the apparent absence of the Y chromosome. Page reasoned that these men must contain a piece of Y chromosome, attached to one of their X chromosomes, that does not show up under light microscopy. Conversely, he reasoned, XY females must be missing a crucial part of the Y chromosome, the part that determines maleness. In both, that crucial piece of Y that defines sex by its presence or absence must contain the TDF gene.

Working with Albert de la Chapelle of the University of Helsinki and Jean Weissenbach of the Pasteur Institute in Paris, Page began several years ago looking for pieces of the Y chromosome in XX men, using DNA probes to detect whether portions of the chromosome are present or missing, a process known as deletion mapping.

These probes are readily identifiable pieces of DNA that Page knew were located on the Y chromosome. When mixed with

David Page

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Margaret Lampert

other DNA, they will seek out and bind to complementary sequences. If this binding, or hybridization, occurred with DNA from the XX men, then Page would know that they carried at least some of the Y chromosome.

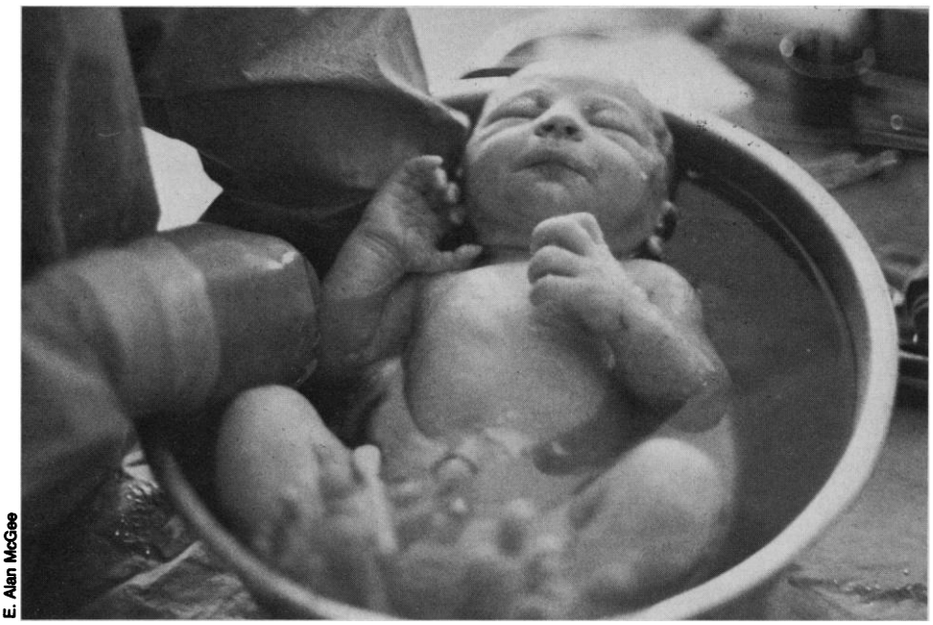
The real insight, Page says, came about a year ago when they found that XX males carry part but not all of the Y chromosome—and that the amount varies from person to person. That piece is on the short arm of the Y chromosome, which gave him a rough chromosomal location for the TDF gene. (This work was also instrumental in showing that the H-Y antigen, at that time the leading candidate, could not be the sex-determining gene because it maps to a different part of the chromosome.)

Page assumes that the XX chromosomal aberration, which occurs in 1 in 20,000 men, arises when something goes amiss during spermatogenesis and the wrong pieces of the X and Y chromosomes are swapped during crossing over. As a result, the father's sperm carries an X chromosome that has gained a small portion of the Y.

The key question, then, for locating the gene, was to determine the lowest common denominator—the smallest piece of DNA that XX men have in common. To find out, Page and his colleagues refined the deletion map, dividing the short arm of the Y chromosome into 13 intervals, or segments, and continued screening XX men. It was this work, published in the 24 December issue of *Cell*, that led Page and his colleagues to the gene.*

Page and his colleagues took a similar tack for XY females, screening their DNA to see which piece of the Y chromosome they were missing—again, looking for the lowest common denominator. Some of these patients contained almost the entire Y chromosome and yet were female. (XY females, however, often have abnormal breast development or do not menstruate.) They found that all of the XY females were missing a piece of the Y chromosome—the same piece that was present in the XX males.

Two patients, in particular, helped narrow the search. One XX male contained only 0.5% of the Y chromosome, about a 250-kilobase stretch of DNA called interval 1A. All the other males contained at least that piece, plus some additional Y DNA. Thus, the gene had to be within that one interval. A 12-year-old girl was the decisive case. She contained 99.8% of the Y chromosome, and lacked only interval 1A2 (a small section of interval 1A) and interval 1B. Analysis of other females suggests that interval 1A2 is the crucial part. Thus, the gene had to be contained within that 140-kilobase stretch. In about 90 sex-reversed patients analyzed to date, says Page, there is an "absolute correlation" between the presence and absence of interval 1A2 and maleness and femaleness.



E. Alan McGee

Sex determination: *The discovery of "sex-switch" genes on the X and Y chromosomes implies that sex determination may be the result of a "gene dosage" effect.*

Within this region Page and his colleagues have pulled out what they think is the sex-determining gene, but without a clue to its biochemical nature that is difficult to prove. To build their case, they looked for a similar gene on the Y chromsomes of other mammals. They detected essentially the same gene is every mammal they looked at—including gorillas, monkeys, dogs, cattle, rabbits, horses, and goats. To do so, they used what Page calls "Noah's Ark blots" because they analyzed a male and female from every species. This "striking degree of evolutionary conservation" makes a strong, if circumstantial case, that this is indeed the TDF gene.

The same gene also shows up in XX male mice, on the small piece of Y DNA they contain, further clinching the case. But they will not be sure, says Page, until they see whether this gene, when inserted into XX mouse embryos, makes them develop as males. Page expects to begin those transgenic mouse experiments soon.

But one gene is not the whole story—sex differentiation is a complex process that seems to involve genes on the sex chromosomes and on the autosomes as well. Thus, what everyone wants to know is where and when the gene acts and what it does.

An answer can be inferred, at least, from an analysis of the nucleotide sequence of the gene, a portion of which Page has determined. From that sequence, they can predict the amino acid structure of the protein encoded by the gene and then use computer analysis to compare that structure to other known proteins. The protein, it turns out, has a unique structure, with repeated sequences, that is remarkably similar to what are known as "finger proteins" because of their shape.

Several finger proteins are known to bind to other genes and regulate the amount of RNA, and thus protein, that they make. And that is a good guess for how the TDF gene works as well, Page says, by turning on and off other genes involved in sex determination.

"We suspect the genes it acts on are essential players in sex determination," says Page. "This protein is clearly setting in motion the entire pathway of sex determination. But all of this is speculation, based on computer analysis," he cautions. "It remains to be proved that this protein is a transcription factor. But we really are excited. It gives us a model for what the gene might do."

Perhaps the most intriguing finding to come out of this work, Page says, is that an almost identical gene is present on the mammalian X chromosome as well, possibly encoding a similar protein. "The sequences are not identical, but the genes could be functionally equivalent." The problem is that sex is supposed to be determined by the Y chromosome. So the immediate task, Page says, is to determine what that gene is doing

* Page's coauthors on this paper, "The sex-determining region of the human Y chromosome encodes a finger protein," are Rebecca Mosher, Elizabeth M. Simpson, Elizabeth M. C. Fisher, Graeme Mardon, Jonathan Pollack, Barbara McGillivray, Albert de la Chapelle, and Laura Brown.

on the X chromosome. Is it involved, directly or indirectly, in sex determination he wonders.

"The X-Y homology is disturbing," says Ulrich Müller of Harvard Medical School, who has also been searching for the gene. "You would not expect the TDF gene to be on both the X and Y chromosomes. You would naïvely expect that it would be just on the Y." The gene on the X chromosome could be nonfunctional, a pseudogene, Müller suggests. But if it is functional, as Page suspects, then this might not be the TDF gene after all.

Page's preference, however, is to throw out the old model, not the gene. In his view, the gene on the X chromosome is functional and is probably involved in sex determination, although he has no evidence for that. He is now trying to incorporate the gene's existence into a coherent model of how sex determination might occur. He has several hypotheses.

One possibility, which Page does not believe, is that the X gene is not involved in sex determination at all. Another possibility is that the two genes work in concert to determine sex, perhaps each encoding a subunit of a multimeric structure. A third model is that the two genes work antagonistically to determine sex—for instance, one could be a negative transcriptional regulator, the other, a positive regulator.

The fourth model, which Page favors because it is the "most outrageous," is that the X and Y proteins are essentially identical and that sex is determined by a dosage effect. This presumes that one X locus is subject to X chromosome inactivation—that is, it is shut off by the other X chromosome. Thus, males would have two active copies of the TDF gene, females only one, and sex would be determined by the total number of expressed genes.

"Other scientists say that this is much too subtle a way to make such an important developmental decision," Page says, "but in invertebrates there is ample precedent." Just such a 2:1 dosage effect determines sex in both nematodes and in *Drosophila*.

"You could also interpret the fact that we found a similar gene on X as evidence that we don't have the right gene at all," says Page, "but I don't actually find that very troubling. Periodically there have been major upheavals in notions about mammalian sex determination. Humans were found to have X and Y chromosomes in 1923. And from 1923 to 1959 it was thought that it was the number of X chromosomes that was sex-determining, and the Y chromosome had nothing to do with it. It may just be time to reshuffle our thinking again." ■

LESLIE ROBERTS

Has a Brown Dwarf Been Found at Last?

The evidence is not ironclad, but it is certainly the most convincing to date; if real, the implications could be cosmic

A recent survey of some 40 white dwarf stars has turned up striking evidence that one of them is accompanied by an orbiting "planet." More precisely, the companion seems to be the first example of a brown dwarf: a dim, star-like object that is not quite massive enough to ignite by thermonuclear fusion.

The discoverers, Benjamin Zuckerman of the University of California, Los Angeles, and Eric E. Becklin of the University of Hawaii, are the first to admit that brown dwarfs have had a checkered history of late. So have extrasolar planets in general; too many people have announced "discoveries" that later turned out to be marginal, or ambiguous, or impossible to confirm. Nonetheless, Zuckerman and Becklin's signal is remarkably clear-cut. And if their interpretation is correct, it will have implications in fields ranging from star formation to cosmology. Astronomer Jesse L. Greenstein of the California Institute of Technology is only half joking when he says, "The implications are literally infinite."

Brown dwarfs have long been something of a paradox for astronomers. On the one hand, it is at least conceivable that brown dwarfs make up much of the universe's so-called dark matter, the invisible ectoplasm that seems to permeate every galaxy and that makes itself felt only by its gravitational influence on the visible stars. Brown dwarfs would certainly be hard to see, since they would be small and would have no way of generating visible light. (They would be roughly the size of Jupiter—11 times the diameter of Earth—and would have masses ranging up to some 0.08 times that of the sun, which is the minimum threshold for thermonuclear burning.) And in theory they should be abundant: a straightforward extrapolation from known stellar populations, where low-mass stars greatly outnumber high-mass stars, suggests that brown dwarfs ought to dominate the mass of our galaxy.

On the other hand, one has the question that the physicist Enrico Fermi once asked about extraterrestrial intelligence: "Where is everybody?" If brown dwarfs are really as ubiquitous as the extrapolations suggest, then why have they not been seen already? At least a few should have shown up as companions to brighter stars. A few more—those that happened to be drifting through interstellar space near the solar system—should have shown up in infrared surveys as warm spots on the sky. (A brown dwarf would retain enough primordial heat from its formation to glow cherry red for billions of years.) And yet not one confirmed example of a brown dwarf has ever been found. Does this mean that they never formed in the first place? Or does the lack of brown dwarfs simply mean that no one has figured out the right way to find them?

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Zuckerman and Becklin's idea was to look for the thermal emission of substellar objects in orbit around white dwarf stars; a typical brown dwarf with a surface temperature of 1000°C or more would then show up as an excess of radiation in the infrared. Zuckerman and Becklin chose to survey white dwarfs not because their quarry was more likely to be found there, but because brown dwarfs would be easier to see there if they did occur. White dwarfs are essentially the embers of normal stars that have exhausted their hydrogen fuel. As such they still tend to be quite hot—10,000 K or more is typical—which means that most of their luminosity comes out at visible wavelengths instead of in the infrared, where a brown dwarf would be brightest. At the same time they are quite small—about the size of Earth—which means that their luminosity is low to begin with. The upshot is that any brown dwarf radiation would emerge with minimal interference.

Today, after surveying some 40 white dwarfs, Zuckerman and Becklin can safely conclude that white dwarf/brown dwarf pairs are not very common. Most of their