that are more credible than currently available projections. ■

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In retrospect

Twenty-five years of the sex-determining gene

The discovery that the gene *SRY* on the mammalian Y chromosome drives testis development marked a turning point in the decades-long quest to understand the genetic underpinnings and evolution of sex determination.

JENNIFER A. MARSHALL GRAVES

It has long been known that a testis-determining factor (TDF) on the Y chromosome kick-starts testis development in humans and other mammals. The testes make hormones, and these hormones make the embryo male. Twenty-five years ago, Sinclair *et al.*[1] reported in *Nature* that TDF was the gene *SRY*. This discovery opened up the surprisingly intricate genetic pathway that determines whether a baby is born a boy or a girl. It also led to an understanding of how genes on the Y chromosome evolved, and of the impact of this key evolutionary event.

Until the 1980s, there was no viable candidate sex-determining gene. Just where was TDF located? What kind of product did it encode? What did it do? During the 1980s, the position of TDF was narrowed down to a small region on the short arm of the Y chromosome, when it was found that some males had XX chromosomes that harboured a small piece of the Y, whereas some females had XY chromosomes that lacked bits of the Y — these added and deleted regions of Y were assumed to contain the TDF sequence. The race was then on to find TDF.

In 1987, the geneticist David Page and his associates[2] identified the first coding gene on the human Y, called *ZFY*. The gene looked like a winning candidate: it was in the right place; it was expressed in the testis; and it was evolutionarily conserved in other placental mammals, such as monkeys, mice, dogs and horses. But in 1988, PhD students in my laboratory[3], Andrew Sinclair and Jamie Foster, mapped *ZFY* to a non-sex chromosome (an autosome) in marsupials, which are a separate branch of mammals. A few months later, it was found[4] that, although *ZFY* is expressed in mouse sperm precursors, it is absent from the other cells of the testis, where a true TDF must be expressed to exert a sex-determining effect.

Sinclair joined a renewed hunt for human TDF in the laboratory of geneticist Peter Goodfellow, using DNA from XY males that had even smaller pieces of the Y than had previously been studied. This was slow and frustrating work, because the Y chromosome is full of repetitive sequences and so specific regions are hard to pinpoint. It was 1990 before they found[1] a small coding gene close to the end of the Y chromosome (Fig. 1). Noncommittally they called the gene *SRY*, for sex region on the Y. The final proof that *SRY* was the TDF came from the discovery of *SRY* mutations in XY females[5] and from the demonstration that adding *Sry* to XX mice was sufficient to induce male development[6]. *SRY* was located on the Y in other placental mammals and, thankfully, even in marsupials[7].

Researchers in the field imagined that identifying TDF would rapidly lead to an understanding of how it worked, and would point to other genes in the sex-determining pathway. But 25 years on, it has become clear that the pathway kick-started by *SRY* is complex, full of checks and balances.

Initially, *SRY* proved a puzzle because it was unlike any known gene. It turned out to be a member of a previously unidentified family, now called the SOX genes. Painstaking biochemical studies of the SRY protein revealed that it bound to a certain DNA sequence and bent it at an angle, presumably to bring other sequences — or the proteins bound to them — into proximity, promoting or inhibiting transcription[8]. The discovery of a different

50 Years Ago

The Royal Society Anniversary Address by Lord Florey, O.M., P.R.S. Perhaps the deployment of Government resources is the modern equivalent of events in the early days of the Society when Fellows contributed—or sometimes did not contribute—a shilling a week towards demonstrating experiments at meetings. There never was enough money … At the moment it is considered to be desirable to give free medicine to all. The application of free calamine lotion to the irritated skins of the populace may be more important than administering to the needs of irritated scientists; but this sort of judgement is in the realm of politics … it has long been the policy of the Society to have symposia and lectures … the popularity of such gatherings has brought difficulties … on one occasion, we had to migrate to the lecture theatre of the Shell Building on the South Bank … one consequence of this peripatetic existence has been that we have had to procure a coffin-like box for the transport of the mace, and I am sure that our original Fellows, and even Charles II himself, might have been somewhat astonished at the adventures of their royal emblem.
From *Nature* 18 December 1965

100 Years Ago

The Romanes Lecture … was a scathing indictment of the ineptitude of the lawyer-politicians who possess a dominating influence on national affairs … To the neglect of science, and the excessive predominance in Parliament and the Government of men with the spirit of the advocate to whom all evidence which will not support their case is unwelcome, Prof. Poulton ascribes the chief mistakes in the conduct of the war.
From *Nature* 22 November 1915

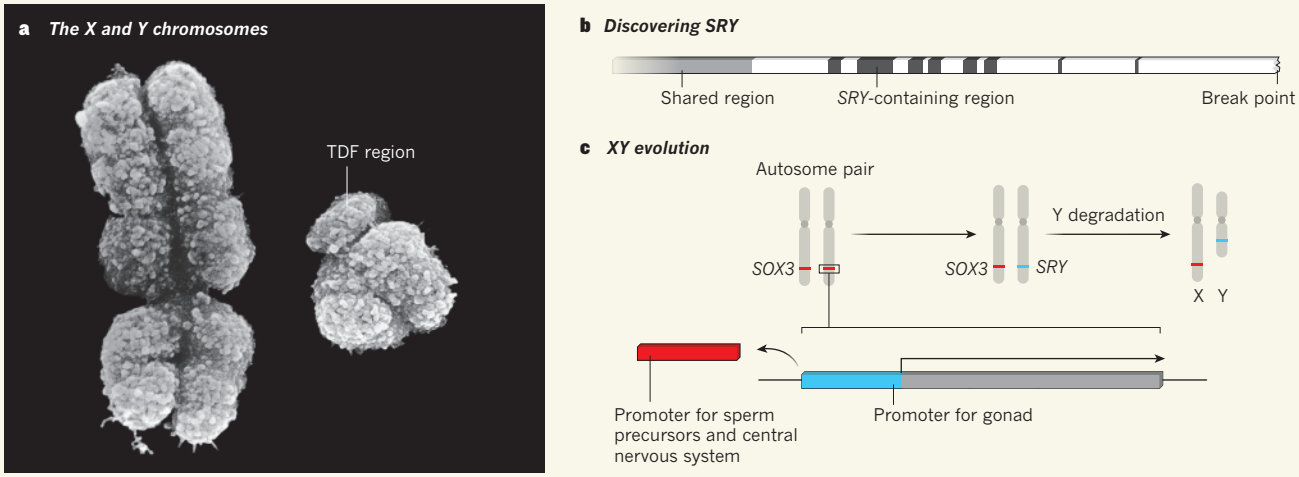

Figure 1 | An evolving understanding of sex. **a**, In humans, sex is based on the presence or absence of the Y chromosome, seen here with its larger partner, X. The testis-determining factor (TDF) that drives male development was known to lie on the short arm of Y, but its identity was a mystery. **b**, In 1990, Sinclair *et al.*[1] found two males with only a small piece of Y, which had been broken and fused to the X. They scoured the 35,000 base pairs between the break points and the region at the tip of the Y that is shared with the X, finding several regions (black) that were specific to the Y. One of these regions contained the TDF gene, *SRY*. **c**, This discovery led to an understanding of how X and Y evolved. The gene *SOX3* was located on a pair of non-sex chromosomes (autosomes) in the ancestors of mammals. A promoter sequence drove expression of *SOX3* in sperm precursors and the central nervous system. The promoter on one copy of *SOX3* was replaced with a sequence that drives expression in the undifferentiated gonad (a tissue that can develop into either an ovary or a testis). This expression pattern allowed the new gene, *SRY*, to direct testis development. Over time, genes not needed for male development were degraded on this chromosome, giving rise to the Y. (Part **b** adapted from ref. 1.)

SOX gene that was disrupted in XY female babies with a severe bone deformity[9,10] revealed that this gene, *SOX9*, is the binding target of SRY protein. *SOX9* is now known to be a master regulator of sex determination throughout the vertebrates.

Studying the mutations that cause sex reversal in humans, mice, goats or dogs (the same pathway is active in all mammals) has proved a successful strategy for identifying many genes in the sex-determination pathway. Gradually, a network of genes that are regulated by, or regulate, *SRY* or *SOX9* has been constructed, and their function tested by mutating the genes in mice[11]. Some genes promote testis formation, some maintain it, and yet others oppose them. This pathway and its control is still being explored. Our improved understanding has helped us both to answer fundamental scientific questions and to diagnose and treat many babies who are born with disorders of sex determination[12].

The other major line of research enabled by the identification of *SRY* was the evolution of sex genes and chromosomes. The hunt for *SRY* in marsupials revealed that mammals have an *SRY*-related gene on the X chromosome, *SOX3*, which was proposed to be the ancestor of *SRY*[13]. This idea is supported by human and mouse data[14] that showed that misexpression of *SOX3* in the undifferentiated gonad (a tissue can develop into either an ovary or a testis, depending on the signals it receives) drives male development in XX embryos. *SRY* probably evolved from *SOX3* when its 5′ region was replaced by a promoter sequence that drove expression in the gonad (Fig. 1).

Although it might seem counterintuitive that the testis-determining factor evolved from the X chromosome, it has since emerged[15] that 20 of the 27 genes on the male-specific part of the human Y evolved from genes on the X. Thus, the Y is basically a degraded X chromosome. This supports the hypothesis that sex chromosomes originate when one member of an autosome pair acquires a sex-determining gene. Nearby genes then also acquire a sex-specific function, crossing over between the chromosome pair is suppressed to keep the male-specific gene package together, and the genetically isolated region on the sex-specific chromosome degrades rapidly[15,16].

"It has become clear that the pathway kick-started by* SRY *is complex, full of checks and balances."

The mammalian XY sex pair was probably defined by the evolution of *SRY*. Vertebrate phylogeny puts the age of *SRY* and the XY pair at between 166 million and 190 million years old. Furthermore, rapid speciation in other lineages that have undergone sex-chromosome turnover raises the possibility that acquisition of *SRY* might have driven the divergence of the egg-laying monotreme mammals from the rest of the mammalian lineage — monotremes have a bizarre, complex sex-determination system that is related to bird sex chromosomes[17].

The future of the Y chromosome is now hotly debated. Evidence suggests that the mammalian Y will disappear in just a few million years if gene loss continues at the same rate as in the past[18]. It has already disappeared in two groups of rodents, and *SRY* has been replaced by another gene from the sex-determining network[19]. The primate Y seems more stable[20], but will eventually erode away. Humans may be in for another round of sex-chromosome turnover — and maybe speciation — if and when *SRY* finally bows out. ■

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