The rise and fall of *SRY*

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Comparisons between species reveal when and how *SRY*, the testis-determining gene, evolved. *SRY* is younger than the Y chromosome, and so was probably not the original mammal sex-determining gene that defined the Y. *SRY* is typical of genes on the Y chromosome. It arose from a gene on the proto-sex chromosome pair with a function (possibly brain-determination) in both sexes. It has been buffeted in evolution, and shows variation in copy number, structure and expression. And it is dispensable, having been lost at least twice independently in different rodent lineages. At the observed rate of attrition, the human Y chromosome will be gone in 5–10 million years. This could lead to the extinction of our species or to a burst of hominid speciation.

Humans and other mammals have a chromosomal system of sex determination. Females have two X chromosomes, and males a single X and a smaller, HETEROCHROMATIC (see Glossary) Y that bears a male-dominant testis-determining factor (TDF). The theory that sex chromosomes arose from autosomes implies that TDF defined the mammalian Y at the dawn of mammal evolution. Its function in triggering testis differentiation is consistent with the expectation that it is a highly conserved transcription factor. However, evidence accumulated over the ten years since the identification of this factor as *SRY* (for 'sex-determining region of the Y') [1] reveals an ephemeral gene, recently recruited from brain development, that changes rapidly, works indirectly, and will soon be consigned to the scrapheap of evolution. As such, *SRY* is a typical Y-chromosome gene.

The Y chromosome and sex

Mammal X and Y chromosomes differ in size and gene content. The X maintains the same 5% of the haploid genome and the same suite of genes in all EUTHERIAN mammals. The 165-Mb human X contains about 1500 genes [2,3], most of which have housekeeping or specialized functions in both sexes. By contrast, the Y chromosome is smaller and almost devoid of genes. The 60-Mb human Y contains only about 50 functional genes (information at www.ncbi.nlm.nih.gov/genome/guide/human) embedded in a morass of repetitive sequence – hardcore junk DNA. At least a half of these Y-borne genes are specialized for sex and spermatogenesis. Uniquely, Y-borne genes vary between species in their presence or absence, copy number, sequence and activity.

Comparative gene mapping and chromosome painting between the three major mammal groups (eutherians, MARSUPIALS and MONOTREMES) has shown that sex chromosomes consist of two regions with distinct origins [4] (Fig. 1). One region of the X is conserved in all mammals and therefore must be ancient. However, another region is on the X in eutherians but autosomal in the other mammals and must have been added recently in the eutherian lineage. This added region, comprising most of the short arm of the human X, shows signs of its recent acquisition because many of the genes do not undergo X INACTIVATION. The Y chromosome, too, contains a region conserved on the Y in all mammals, and an added region that is on the Y only in eutherians [5].

The observation that the ancient part of the sex chromosomes is shared between all three mammalian groups means that the mammalian Y chromosome must have come into being before monotremes diverged from the therians (marsupials and eutherians) 170 million years (Myr) ago.

Although the X and Y are so different in size and gene content, they share some homology. A homologous 'PSEUDOAUTOSOMAL' REGION (PAR) pairs and recombines at meiosis, and many genes in the differential region of the Y have copies on the X. This supports the hypothesis that X and Y chromosomes evolved from an autosomal pair, an idea proposed by Susumo Ohno [6], originally to explain gradations in sex chromosome differentiation in snake families. The hypothesis states that a pair of autosomes became proto-sex chromosomes when a new

Glossary

Eutherian: 'Placental' mammals, such as human and mouse.
Heterochromatin (heterochromatic): Chromatin that is cytologically distinguishable because of its altered condensation and staining properties.
Hitchhiking: A proposed mechanism for Y degeneration. A favourable new mutation on a Y chromosome results in the rapid selection of the Y that carries it. Any mutations and deletions contained on the Y being selected then 'hitchhike' to fixation.
Marsupial: Branch of mammals often (but not always) pouched, that diverged from eutherians 130 million years ago. Together with Eutheria, they form mammalian Subclass Theria.
Monotreme: The third major group of mammals, consisting of the egg-laying platypus and echidna. They diverged from therians about 170 million years ago.
Müller's ratchet: A mechanism proposed to explain Y degeneration. At each turn of the ratchet, Y chromosomes with the fewest mutations are accidentally lost from the population, and in the absence of recombination, can never be regenerated.
Pseudoautosomal region (PAR): The small region of homology between the X and Y chromosomes that pairs and recombines at male meiosis. In humans, the X and Y regularly pair in the 2.6-Mb PAR1 on the short arms, and there is also a small long arm PAR2.
X chromosome inactivation: Stable and heritable epigenetic silencing of one X chromosome in the somatic cells of female mammals.

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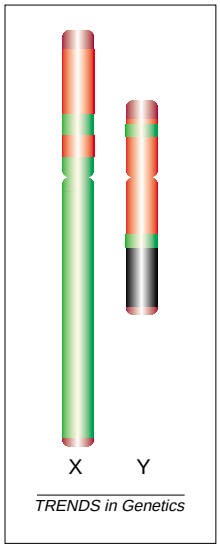

Fig. 1. The ancient (green) and added (red) regions of human sex chromosomes. Regions homologous to marsupial and monotreme sex chromosomes (green) must be >170 million years old. Regions homologous to marsupial and monotreme autosomes (red), including the pseudoautosomal (dark red) must have been added to the X and Y <130 million years ago. Note that almost nothing remains of the ancient Y.

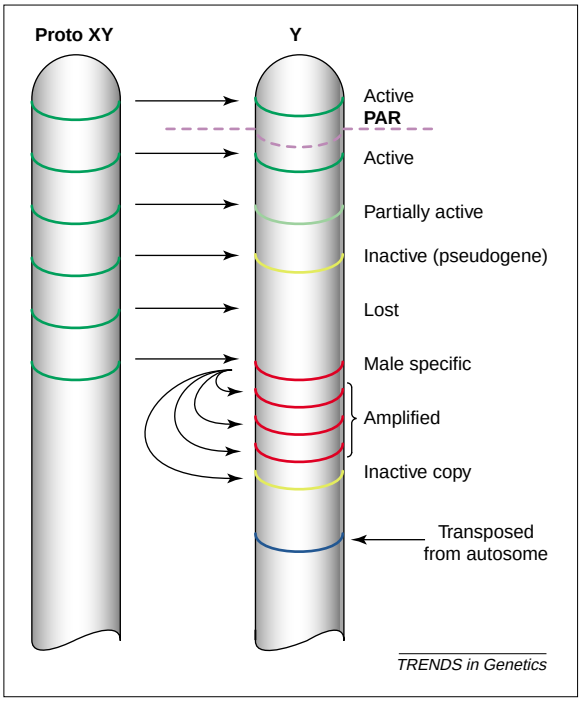

Fig. 2. The origins of genes and pseudogenes on the Y chromosome. Genes on the original proto-XY homologous pair (green) remain unchanged in the pseudoautosomal region (PAR) of the Y. As the Y degrades, they can remain active and complement function (green), become partially active (pale green), become an inactive pseudogene (yellow) or, like most, be completely lost. Some (e.g. *SRY*) are modified to acquire a male-specific function (red). These are frequently amplified and many copies are inactivated. A few genes might be transposed from autosomes (blue); these can also be modified, amplified and inactivated. Green and yellow genes comprise Class I, whereas red and blue genes have been claimed to represent a separate Class II.

sex-determining allele evolved on one partner. Other alleles with a function in that sex then accumulated near the sex-determining locus. It therefore became advantageous to suppress recombination between the proto-sex chromosomes, so that the whole region could be inherited as a sex-specific package. Further accumulation led to further suppression of recombination. Within the non-recombining region, mutations, deletions, insertions and amplification led to gene inactivation and accumulation of repetitive elements, permitted by processes of drift (MÜLLER'S RATCHET) and HITCHHIKING with a favourable allele [7]. Thus the acquisition of a sex-determining allele by the Y chromosome triggered a continuing process of degradation.

We assume that the initiating event that defined the proto-Y chromosome was the acquisition of TDF. Accumulation of other male-specific genes then led to suppression of recombination, and the degradation of the Y chromosome. When and how did this happen?

To discover the origin of the Y chromosome, we must compare sex-determining systems between mammals and other vertebrates. Among a confusing variety of chromosomal, genetic and environmental sex-determination systems, we find no obvious homology to the mammal system [8]. Birds and snakes have well-differentiated sex chromosomes, but it is the female that has a single large Z chromosome and a smaller, heterochromatic W, whereas males have two Z chromosomes. Sex seems to be determined both by Z dosage and a W effect, perhaps by dosage of the *DMRT1* gene [9] on the Z, abetted by a W effect [10]. There is contradictory evidence as to whether the Z chromosome is dosage compensated in males [11]. Gene mapping shows that the mammal XY and bird ZW pairs are completely unrelated [12], implying that they evolved independently from different autosomal pairs. This means that the Y chromosome evolved after the divergence of mammals from reptiles 310 Myr ago.

The mammalian Y chromosome is therefore 170–310 Myr old.

The origin of Y-borne genes

Ohno's hypothesis predicts that genes on the Y chromosome are all relics of genes that were on the ancient proto-sex chromosome or the added region. They should all therefore have X-borne homologues, whose sequence and function have remained largely unchanged.

Most of them do. However, several multicopy testis-specific genes on the Y that appeared to have no X homologue were placed in a discrete 'Class II' of male-advantage genes [13] that were acquired by a 'selfish Y' [14] and amplified. Two genes, *CDY* and *DAZ*, fit this description, being on the Y only in humans, presumably as a result of recent transposition from autosomal homologues. The *DAZ* gene is part of a larger (78-kb) unit that was transposed from chromosome 3, duplicated, inverted, then a palindromic sequence further amplified [15]. *CDY* was retroposed from a gene on chromosome 13 [16].

However, X-borne partners have been discovered for at least some genes that are multicopy and testis-specific, such as the candidate spermatogenesis gene *RBMY* [17]. The distinction between two discrete classes of Y-borne genes also breaks down when other species are considered, because several genes such as *ZFY* [18] belong to Class I (single copy with X-linked homologues) in humans, but are Class II (testis-specific and multicopy) in rodents.

It therefore seems likely that many or most Y-borne genes represent a spectrum of degradation and specialization, from full homology to complete loss (Fig. 2). Class I genes are simply those at the beginning of this process. The first stages of degradation are seen in genes (such as *RPS4Y*) that are partially active [19]. Further degradation left numerous pseudogenes on the Y with active homologues on the X such as *STS* [20]. The last stage is represented by >1400 X-specific genes whose partners were lost from the Y. Some Class II genes, such as *DAZ* and *CDY*, have been selfishly acquired from autosomes, but many Class II genes arose from genes on the proto-XY and acquired a selectable male-specific function. Many male-specific genes were amplified in a desperate race to stay ahead of

inexorable degradation [21]. They have been retained, at least for a few million years, but could ultimately be superseded. For instance, a putative spermatogenesis gene *UBE1Y* is Y-borne in mouse and marsupials but not primates [22].

So how did genes on the Y acquire a male-specific function? Most X-borne homologues are expressed widely in both sexes and presumably serve housekeeping functions, so the male-specific function must have been acquired after the Y became isolated. For instance, *RBMY*, thought to have a role in spermatogenesis because it is testis-specific and lies within deletions that cause azoospermia, has a homologue on the X, *RBMX*, which is ubiquitously expressed and lies within deletions that cause mental retardation [17]. How was this putative brain-development gene reconfigured into a spermatogenesis gene? The most glaring sequence change to *RBMY* is the acquisition of three extra exons by exon amplification, but it is more likely that the crucial changes involved tissue-specific control. Similarly, the testis-specific *ATRY* in marsupials has a ubiquitously expressed X-borne homologue *ATRX* [23]. Humans and mice possess no Y copy, and mutations in the ubiquitously expressed X-specific *ATRX* produces a phenotype (α-thalassemia, mental retardation and sex reversal) suggesting a general transcription factor [24]. Again, a gene with wide effects has been re-shaped into a male-specific (possibly testis-differentiating) gene on the Y.

Against this background of degradation and specialization of Y-borne genes, I now consider the testis-determining factor itself, to demonstrate that *SRY* is a typical Y-borne gene.

SRY and its relatives

After several false starts, the human testis-determining gene was isolated by positional cloning [1]. *SRY* had homologues on the Y chromosome in other eutherians [25] and (unlike earlier contenders [26]) even marsupials [27]. Its function in testis determination was verified by detecting *SRY* mutations in human XY female patients, and by transgenesis in mice [28].

SRY was not the obvious transcription factor that might be anticipated from its action as a male-dominant trigger for testis differentiation. It showed similarities to the high mobility group proteins that are architectural factors involved in chromatin structure. They share an 'HMG box', a protein domain that binds to DNA at a target sequence and bends it through a specific angle, bringing non-adjacent DNA sequences, or the proteins bound to them, into some kind of productive interaction.

There are now more than 20 genes that belong to the same family as *SRY* [29]. These *SOX* (for '*SRY*-like HMG-box-containing') genes have a variety of important functions in development and are highly conserved between animal species. One, *SOX9*, has a conserved role in testis determination, as well as in chondrogenesis.

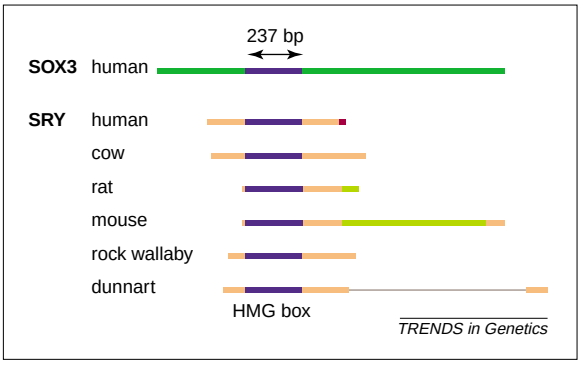

Fig. 3. The genomic structure of *SRY* coding regions in different species, compared with the conserved ancestral gene *SOX3*. The HMG box (blue) is well conserved, but *SRY* flanking regions (orange) are hard to align between different species, and differ from *SOX3*. Striking differences are the PDZ domains (crimson) of primate *SRY* [44], a CAG-rich domain (light green) of different lengths in rodents [43], and a *de novo* intron (grey) in the dunnart *SRY* [49].

SRY is an atypical family member, being poorly conserved inside the box, and impossible to align outside it (Fig. 3). Most sex reversing mutations of human *SRY* lie within the HMG box, and the products of mutant proteins either bind poorly or bend DNA though the wrong angle [30]. This suggests that all the activity of *SRY* lies in its capacity to bind and bend DNA, controlling chromatin structure to influence the activity of an unknown target gene [31].

It was expected that the male-dominant TDF would be specific to the Y chromosome. It was therefore surprising to detect a gene closely related to *SRY* on the X chromosome [32]. One of five *Sry*-related genes detected in mice by Southern blotting [25], *SOX3* was subsequently mapped to the X in marsupials, humans and mice. Phylogenetic analysis shows that *SOX3* is the gene most closely related to *SRY* [29], supporting the hypothesis [32] that *SOX3* was the ancestor of *SRY*. *SOX3* is expressed largely in the central nervous system of mouse embryos. However, there is some expression in the developing testis in mouse [33], though not in marsupial [34], and it binds, with other *SOX* genes, to the steroidogeneic factor 1 (*SF1*) gene that is essential for endocrine proliferation [35]. On the basis of its functional homology and interactions, it has been proposed that *SOX3* is involved in testis determination by interacting with *SOX9* and *SRY* [36].

Thus, even the sex-determining gene on the Y evolved from a gene on the X with more general functions in both sexes. To explain how a brain-determining gene could evolve into a testis-determining gene, I will chart the rise of *SRY*.

The rise of *SRY*

When and how did *SRY* evolve? Was this a cause or a consequence of Y chromosome differentiation?

If the acquisition of TDF defined the Y chromosome as the hypothesis predicts, we would expect *SRY* to be the first gene differentiated, 170–310 Myr ago. The absence of a sex-specific *SRY*

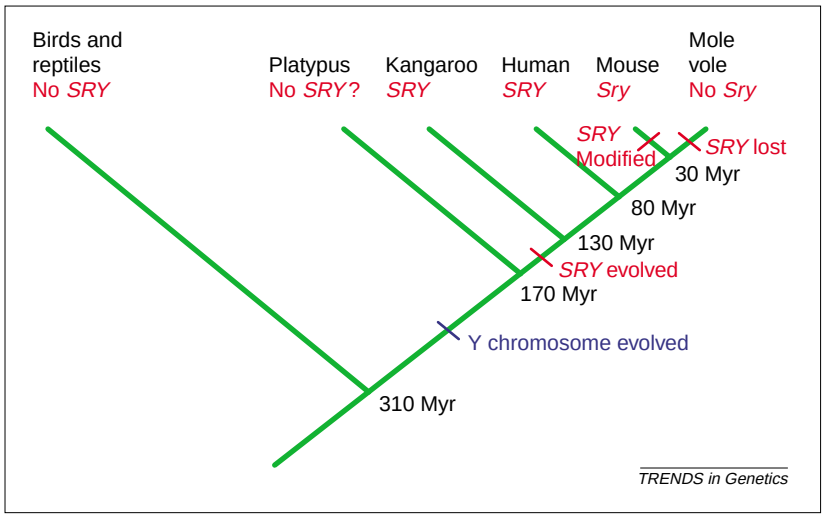

Fig. 4. Timescale of evolution of the mammalian Y chromosome and *SRY*.

gene in birds and reptiles [37] implies that *SRY* is less than 310 Myr old, and the presence of *SRY* in marsupials as well as eutherians [27] means that it is at least 130 Myr old. However, all attempts to detect a sex-specific *SRY* gene in monotremes, by Southern analysis and PCR of platypus and echidna DNA, using all manner of probes and primers, have failed, and two related *SOX* genes have proved to be autosomal (P. Kirby, unpublished). If monotremes truly lack *SRY* (a difficult thing to establish with negative evidence), this implies that *SRY* arose 130–170 Myr ago, 40 Myr after the Y chromosome got its start (Fig. 4), or alternatively, was lost in the monotreme lineage. The evolution of *SRY* might therefore be a consequence, not a cause, of X–Y differentiation.

If *SRY* was a therian invention, some other gene on the ancient Y must have served as the original trigger for Y degradation. Three candidates present themselves, *DAX1* on human Xp is sex reversing when duplicated [38], so two doses of this gene could have determined femaleness in an ancestral mammal. However, *DAX1* lies within the X added region, so was not on the original proto-Y [39]. An alternative is *ATRX*, which lies in the ancient region of the X and is required for testis differentiation [24]. This gene has no Y homologue in humans or mice, but it does in marsupials [23] (we don't know about monotremes), so it was presumably present on the ancestral mammal Y and subsequently lost in eutherians.

The third possibility is *SOX3*, the gene from which *SRY* evolved. An attractive possibility is that *SOX3* originally functioned in male determination by controlling the activity of *SOX9*. A null allele of *SOX3* could have set up a 2:1 dosage difference between homozygotes (females) and heterozygotes (males). Truncation of this allele then turned it into *SRY*, an efficient inhibitor of *SOX3*, leading to a much more robust male-dominant form of testis determination that would be strongly selected for [36].

It is easy to explain how the brain-determining *SOX3* could evolve into the testis-determining *SRY*. The genes have an overlapping expression profile. In mice, *Sox3* is expressed largely in the central nervous system, but has some expression in the genital ridge [33] at the same time as *Sry* [28], which is expressed largely in the genital ridge, but has minor expression in the brain [40]. *SRY* is similar to *SOX3* within the HMG box, but lacks its 5′ and 3′ domains. Stripped of these non-HMG-box functions, *SRY* could act as a competitive inhibitor of *SOX3*, because it binds to the same target sequence. In the same way, *SOX9*, when stripped of its 3′ sequence, turns from a transcriptional activator into an inhibitor [41].

A discomforting aspect of *SRY* is its remarkable differences in expression, structure and function between species. Whereas mouse *Sry* is transcribed largely in the embryonic genital ridge at the time of testis determination [28], human *SRY* is widely expressed in the embryo and transcribed in adult testis [1], and marsupial *SRY* appears to be ubiquitous [42]. We do not understand what, if anything, these different expression patterns mean.

Moreover, *SRY* structure and function differs between mouse species and other mammals (Fig. 3). Mouse *Sry* has a long 3′ GAC-repeat region that codes for a glutamine-rich C-terminal protein domain [43]. Transgenesis with various deleted constructs shows that this tail is essential for function. Yet, it is absent from human *SRY*, which instead has two PDZ domains that bind protein [44]. It is suggested that the glutamine-rich domain is a recent evolutionary advance allowing direct binding to a target protein rather than relying on protein–protein binding. A recent amplification is supported by the observation that rat and other *Mus* species have glutamine-rich regions of various lengths.

Not all change in *SRY* is for the best. The perplexing finding that, as well as making functional linear transcripts, mouse *Sry* is transcribed into an untranslateable circular RNA in adult testis and several embryonic tissues [45] is more likely to reflect a by-product of a local inversion within a repetitive structure, rather than a brilliant evolutionary advance.

This variation implies that *SRY* structure and function evolves very rapidly. In this, it is similar to several other genes on the Y chromosome. For instance, *RPS4Y* sequence evolved four times as rapidly as its X-borne partner. Human *ZFY* is ubiquitously expressed and could act as a general transcription factor, complementing the action of its X-linked homologue. It is not subject to inactivation because both males and females have two active alleles [18]. However, the duplicated mouse *Zfy* genes are gonad specific, and the ubiquitously expressed *Zfx* is inactivated. This suggests that rodent *Zfy* acquired a male-specific function and no longer complements the general function of *Zfx*.

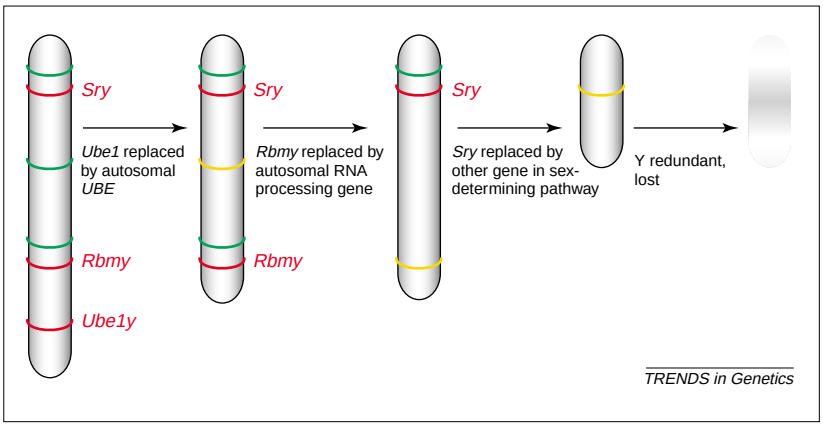

Fig. 5. The loss of the Y chromosome and the *Sry* gene in mole voles (genus *Ellobius*). The ancestral rodent Y (left) retained several genes that are crucial for male differentiation, such as *Sry*, *Rbmy* and *Ube1y* (red), as well as other genes that still complemented their X-borne partners (green). These were all lost (in arbitrary order), and their functions replaced by genes on autosomes or the X. Eventually, the entire Y became redundant and was lost (right).

We can therefore regard *SRY* as a typical Y-borne gene. It arose as the consequence of Y degradation, and continues to evolve rapidly, much as other genes on the Y.

The fall of *SRY*

Most genes on the original Y chromosome, and even those on the added region, were lost over the past few hundred million years. Even genes (such as *Ube1y*) with a function in male differentiation eventually lost the struggle for survival on the primate Y [22]. Thus acquisition of a useful male-specific function might prolong the life of a gene on the Y – but not forever. Could *SRY* also disappear? And if it does, what will happen to the human race?

SRY can indeed disappear. It has done so in two species of the mole vole *Ellobius* [46]. In *E. lutescens*, both sexes are XO, and in *E. tancrei*, both sexes are XX, with one X inactive. A third species, *E. fuscocapillus*, has perfectly normal XY males and XX females. For many years it was thought that the sex-determining and differentiating genes on the Y had merely been translocated to another chromosome in the complement. However, it is now clear that although the *E. fuscocapillus* Y bears quite a normal *SRY* and *ZFY*, there is no *SRY* or *ZFY* in *E. lutescens* or *E. tancrei*. Evidently a new sex-determining system took over in a common *Ellobius* ancestor.

How could *SRY* be lost without creating a crisis of sexual identity? Presumably its controlling function was usurped by a gene elsewhere in the genome, probably another gene in the sex-determining pathway. There are several obvious candidates, including *SOX9*, *DAX1*, *DMRT1* and *ATRX*. So far, only *SOX9* has been exonerated [47], and it remains possible that changes of expression in the hundreds of other genes (mostly unknown) in the pathway, made *SRY* redundant. Identifying the new sex-determining gene in mole voles will clarify how one sex-determining system succeeds another, as well as establishing another step in the normal human sex-determining pathway.

And how could the entire Y chromosome have been lost in mole voles without imposing infertility on Y-less males? This surely must have happened in stages (Fig. 5), in which sex and spermatogenesis genes were lost and each function replaced by a gene on the X or an autosome. The loss of each gene would further destabilize the Y chromosome, making loss even more likely.

For instance, spermatogenesis genes could have been lost from the Y first, and their functions usurped by autosomal homologues. We know that this has happened quite recently in the loss of *UBE1Y* from the primate Y, perhaps after its function in spermatogenesis was taken over by another ubiquitin activating enzyme. In the same way, the function of *RBMY* in RNA splicing could have been taken over by a related autosomal gene, perhaps one of the testis-specific *RBMX* retroposons described recently [48].

If this happened in rodents, it could happen again in other mammals. What are the prospects for human *SRY* and the human Y chromosome?

The future of the human Y chromosome and *SRY*

The evidence suggests that loss of genes on the Y is only a matter of time and chance. *SRY* is typical of Y-borne genes, which are created when an autosome takes on a sex-determining function, are stochastically degraded, mutated and amplified during their lifetime, and are ultimately lost.

So how long have we got? A simplistic calculation of the rate at which genes were deleted from the Y can be made from the number of genes that disappeared from the conserved and the added regions over the past few hundred million years. The ancient part of the Y was once homologous to the X conserved region, which contains about 1000 genes. Only four survive. Thus more than 995 genes have been lost since the Y began to differentiate 170–310 Myr ago, giving an attrition rate of 3–6 genes per Myr. Likewise, only about 20 genes remain on the Y added region, out of an original 500. Thus 480-odd disappeared over the last 80–130 Myr, giving an estimate, again, of 4–6 genes per Myr. At this rate, the human Y chromosome might last another 10 Myr.

Rashly assuming that our species lasts that long, what will become of us when the Y chromosome runs out of puff? Will human sex and fertility decline to zero, leading to our extinction? A more likely scenario is that first fertility genes will go, one by one, as their function is replaced by autosomal genes, then *SRY* will be replaced by another gene in the sex determining pathway. Accumulation of new variants at the site of this new sex-determining gene will start the whole process of sex chromosome differentiation all over again. Maybe this will happen more than once, independently. Populations with different sex-determining genes will not have much luck interbreeding, so this may well prove a speciating event that separates two new hominid species from us, their parent population.

Conclusions

The testis determining factor, *SRY*, is typical of genes on the degenerating Y chromosome. It was probably not the original mammalian TDF that defined the Y chromosome, but was itself a product of Y degeneration. Like other genes on the Y chromosome, it evolved from a gene (*SOX3*) on the original proto-sex chromosome pair. It was diverted from its original, possibly brain-determining, function in both sexes by truncation, leaving a stripped down DNA-binding HMG box that could have turned it into an efficient repressor of other *SOX* genes. And like other genes on the Y chromosome, even those with crucial male-specific functions, it can be lost, and its function taken over by other genes in the sex determining pathway. At its average rate of degeneration, we can predict that the human Y, and the human *SRY* gene, could last only another 5–10 Myr. Its demise may presage the end of the human race, or precipitate a burst of hominid speciation.

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