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The Evolution of Mammalian Sex Chromosomes and the Origin of Sex Determining Genes [and Discussion]

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The evolution of mammalian sex chromosomes and the origin of sex determining genes

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SUMMARY

Mammals have XX female:XY male chromosomal sex determination in which a small heterochromatic Y controls male development. Only a few active genes have been identified on the Y, including the testis determining factor *SRY* and candidate spermatogenesis genes. These genes, as well as several pseudogenes, have close relatives on the X, confirming that the Y was originally homologous to the X, but has been progressively degraded. We used comparative gene mapping of sex chromosomes from the three major groups of extant mammals (eutherians, marsupials and monotremes) to deduce how the X and Y evolved from a pair of autosomes, and how *SRY* assumed control of sex determination. We found that part of the X, and a corresponding region of the Y chromosome, is shared by all mammals and must be very ancient, but part of the X (and Y) was added quite recently. I propose that a small original X and Y were enlarged by cycles of autosomal addition to one partner, recombination onto the other and continuing attrition of the compound Y. This addition–attrition hypothesis predicts that the pseudoautosomal region of the human X is merely a relic of the last addition, and that the gene content of the pseudoautosomal region may well differ in different mammalian lineages. The only genes which remained active on the conserved or added regions of the Y were those, like *SRY*, that evolved functions in male sex determination and differentiation distinct from the general functions of their X-linked partners. Although the vertebrate gonadogenesis pathway is highly conserved, its control circuitry has probably changed radically and rapidly in evolution.

1. INTRODUCTION

Dobszhansky wrote that 'nothing in biology makes sense, except in the light of of evolution' (Ayala 1977). Previous chapters of this volume have attempted to make functional sense of the action of *SRY* and other genes involved in mammalian sex determination. The inconsistencies and intricacies these studies have revealed suggest that the molecular biology of sex determination may provide a good illustratation of Dobzhansky's aphorism.

Mammals have XX female:XY male chromosomal sex determination in which a small heterochromatic Y controls male development. The first identifiable step in the sex determination pathway is testis determination, which is regulated by a Y-borne 'testis determining factor' (TDF), identified a few years ago as the *SRY* gene. Subsequent steps in establishing the male somatic phenotype are controlled by testicular hormones, and are independent of the Y. Other genes on the Y are expressed in testis and may have a role in spermatogenesis. The isolation of the TDF gene promised an entry point into the testis-determining pathway, leading to an understanding of sex determination, and providing the first insight into genetic control of mammalian organogenesis. However, five years after cloning and characterizing the *SRY* gene, its role is still unclear. The genetics of mammalian sex determination seems more complex than was appreciated, perhaps reflecting the tortuous evolutionary route by which genes on the Y took on their male-specific functions.

Here I will develop the hypothesis that genes on the Y chromosome, including *SRY*, are relics of a process by which the Y has evolved from the X by progressive additions and attrition. Genes on the Y are therefore either dispensable, and are in various stages of degradation, or have been selected because they have acquired a male-specific function in sex determination or spermatogenesis.

2. MAMMALIAN X AND Y CHROMOSOMES

In eutherian ('placental') mammals, the X chromosome represents about 5% of the haploid genome, and bears some thousands of genes coding for a fairly standard mix of housekeeping and specialized functions. The gene content of the X is almost completely conserved between species (the best studied being human and mouse), a feature ascribed to its participation in a chromosome-wide inactivation mechanism, which ensures dosage compensation.

The Y chromosome is smaller than the X (only 2% or 3% of the haploid genome), and recombines with it only over a restricted pseudoautosomal region (PAR). The Y is composed largely of repeated sequences, and

Table 1. *Genes on human Y and their counterparts on the X discussed in this review (references in Graves 1995)*

(Human gene nomenclature is used throughout this review except where mouse genes (noted in lower case) are specifically discussed.)

locus symbol	gene name/function	homologue on human Y	homologue in other mammals
C2F2RA	colony stimulating factor 2 receptor	pseudoautosomal	autosomal in mouse
IL3RA	interleukin 3 receptor	pseudoautosomal	autosomal in mouse
ANT3	adenine nucleotide translocase 3	pseudoautosomal	autosomal in prosimians
PDBX	gene spanning pseudoautosomal boundary (= Xga?)	pseudogene PDBY	Y sequence in great apes
STS	steroid sulphatase	pseudogene *STSP*	pseudoautosomal in mouse, autosomal in marsupials and prosimians
KAL	adhesion molecule (Kallman Syndrome)	pseudogene *KALP*	Y sequence only in apes, old world monkeys, undetectable in mouse
AMELX	amelogenin (tooth enamel)	actively *AMELY*, but low transcription	pseudogene in mouse, autosomal in marsupial and monotreme
ZFX	zinc finger protein	active *ZFY* gene, wide expression	active Y genes in eutherians, testis-specific in mouse, autosomal in marsupials and monotremes
DAX1	dosage sensitive sex reversal-AHC critical region on the X	—	—
UBE1X	ubiquitin activating enzyme 1, spermato-genesis factor?	no Y copy in primates	active Y copy in all other therians
SMCX	homologue of selected mouse cDNA on Y (HYA?)	active SMCY gene, wide expression	active Y allele in most therians
RPS4X	ribosomal protein subunit 4	active *RPS4Y* gene, but low transcription	no Y gene in mouse
SOX3	SRY-like HMG box containing gene 3	SRY (testis determining factor)	SRY on Y in therian mammals, variable expression
—	Y-borne RNA-recognition motif	YRRM1	Autosomal homologue, Y homologues in other mammals
—	testis-specific protein, Y-encoded	TSPY	homologue repeated on bovine Y
—	arginosuccinate synthetase pseudogene	ASSP6	autosomal homologues
—	actin-like pseudogene	ACTGP2	autosomal homologue

the only functions originally ascribed to it were that of testis determination (TDF) and a male-specific minor histocompatibility antigen (HYA). However, a dozen or so genes and pseudogenes have now been mapped to the human and/or mouse Y. Some lie in the PAR, and others are distributed within the euchromatic portion of the Y, which also contains functions in spermatogenesis identified by deletion analysis (Ma *et al.* 1993). Genes on the Y provide an insight into the origin of the Y chromosome and its function in sex determination and differentiation.

Of the dozen or so genes on the human Y chromosome, most have relatives on the X (see table 1). Exceptions are processed pseudogenes, and repeated genes like *TSPY* and *YRRM1* whose origins are unclear. The mouse Y contains a somewhat different set of pseudoautosomal and X-Y shared genes. Over several species, the gene content of the PAR is inconsistent, and genes on the differential region of the Y are remarkably variable in their presence, numbers and activity.

The activity of X-linked genes with partners on the Y is also inconsistent. As might be expected, human X-Y shared genes are not subject to X inactivation; however, the observation that most X-Y shared mouse genes are inactivated (Adler *et al.* 1991; Ashworth *et al.* 1991; Agulnik *et al.* 1994*a*) suggests that the functions of X and Y-borne partners do not complement. *STS* is anomalous in both species, being 2X active in human although it has no active Y allele, whereas mouse *Sts* is at least partially inactivated, although it is pseudoautosomal. Thus, deletion or change in function of a Y-borne gene is correlated with recruitment of its X-borne partner into the X inactivation system, although the exceptions suggest that dosage differences can be tolerated over a few million years.

Genes on the Y chromosome therefore seem to represent a small, non-identical but overlapping subset of genes on the X. Some, evidently expendable, genes seem to be dead or dying, while others appear to serve a male-specific function which ensures their survival over long periods of evolutionary time (e.g. *UBE1Y* is on the Y in mouse and marsupial (Mitchell *et al.* 1992). The cloning and characterization of the *SRY* gene, and the investigation of related genes, suggests that the *SRY* gene itself acquired its testis-determining function in this haphazard manner.

The human *SRY* gene, cloned from a minimum sex determining region proximal to the PAR, detects homologues on the Y in males of a wide range of placental and marsupial mammals (Sinclair *et al.* 1990; Foster *et al.* 1992). This small, intronless gene codes for

a protein with homology to an 80 amino acid DNA binding region ('HMG box') shared by many proteins, including the high mobility group. The identity of *SRY* as the testis determining factor was demonstrated by mutational analysis (Hawkins *et al.* 1992), and transgenesis for mouse *SRY* (Koopman *et al.* 1991). Biochemical studies of *SRY* action showed that *SRY* protein binds to DNA at a preferred 6-base consensus target sequence, and introduces specific bends, which might bring other sequences, or proteins bound to them, into juxtaposition required for activity (Ferrari *et al.* 1993; Harley *et al.* 1992). However, it is still quite unclear how this action precipitates testis differentiation, what other genes *SRY* interacts with and even whether it is an activator or a repressor. Unusual for a gene with such a critical function is the poor homology between human, mouse and marsupial *SRY* within the HMG box, and complete absence of homology outside it, suggesting that the only activity of *SRY* is coded by the box itself. Another deepening mystery is the species difference in *SRY* expression patterns. *SRY* is expressed specifically in genital ridge in mouse, but has wide expression in embryonic and adult human and marsupial tissues (Clepet *et al.* 1994; Harry *et al.* 1995), suggesting that *SRY* may retain another (original?) function as well as its sex determining role.

A better understanding of *SRY* function may be gained by studying the evolution of *SRY* and its relatives. Initially it was reported that the human *SRY* probe detected several related '*SOX*' (for *SRY*-like HMG box containing) sequences (Sinclair *et al.* 1990; Gubbay *et al.* 1990). This family includes more than 20 representatives, highly conserved between species within and outside of the box, with important general functions in development of, for instance, central nervous system, in both sexes (Collignon & Lovell-Badge 1993).

The relation of *SRY* to these *SOX* genes became clearer with the discovery that the *SOX* gene most closely related to *SRY* lies on the X chromosome (Foster & Graves 1994). The finding that *SRY*, like other cloned genes on the Y, has a related sequence on the X suggested that this *SOX*3 gene was present on the original proto-X and Y, and that the Y-borne allele, once isolated from recombination, took on a testis-determining function as the *SRY* gene. The stripped-down box of the *SRY* gene could be a degraded version of *SOX*3, and its action may be to repress, rather than to activate another gene in the pathway (Graves & Schmidt 1992; McElreavey *et al.* 1991), perhaps by competitively inhibiting another gene product (*SOX*3??) which shares its DNA binding domain. *SRY* could even be part of a long chain of command, which would limit the information *SRY* can yield about the fundamental steps of gonadogenesis.

There are likely to be many other genes involved in mammalian sex determination, but the male development of XX mice transgenic for *SRY* excludes the possibility that any of these lie on the Y. Several autosomal mutants are known to cause sex reversal in man or mouse (Eicher 1988), and a gene *DAX1* has recently been cloned from human Xp, which, in duplicate, suppresses male determination in XY fetuses (Zanaria *et al.* 1995). Of particular interest to the question of *SRY* function is the recent discovery that *SOX*9, which is closely related to *SOX*3 and *SRY*, is mutated in campomelic dysplasia (Foster *et al.* 1995), an autosomal dominant disorder associated with sex reversal. Expression of mouse *Sox9* is associated with chondrogenesis (Wright *et al.* 1995), but it is intriguing to speculate whether it also has a normal function in sex determination, or whether it interacts in some way with *SRY* and/or *SOX3*.

Deletions of regions of the human and mouse Y produce normal but sterile males, suggesting that some of the genes involved in meiosis and sperm production map to the Y chromosome independently of *SRY*. At least two different spermatogenesis functions have been mapped to the long arm of the mouse Y (Burgoyne 1993), and a spermatogenesis factor has been mapped to a region of mouse Yp that contains *Ube1y*, *Smcy* and *Zfy* (Mitchell *et al.* 1991; Agulnik *et al.* 1994*b*). The region of human Yq deleted in men with azoospermia contains many copies of a gene *YRRM1* (Ma *et al.* 1993), although deletions of other regions are also associated with azoospermia. Another gene, *TSPY*, also repeated on the human and bovine Y, is expressed only in testis and is a candidate for a function in spermatogenesis (Manz *et al.* 1993).

There is variation between species in the presence, numbers and activities of these putative spermatogenesis genes which may connote different functions. For instance, *ZFY* is unique in humans but duplicated in mouse and much amplified in other rodents; it is expressed ubiquitously in humans but is testis-specific in mouse. *Ube1y* is present on the Y in most eutherians and marsupials, but is absent from primates. It is hard to explain such variation in genes apparently critical in reproduction.

Comparisons between the Y chromosomes of eutherian mammals therefore suggest that the genes on the Y chromosome represent a rather random subset of genes on the X. The Y can be regarded as a degraded X, and genes on the Y as relics of this attrition process. Wider comparisons may permit us to infer the beginnings of this process.

3. COMPARATIVE MAPPING AND THE ADDITION/ATTRITION HYPOTHESIS FOR SEX CHROMOSOME EVOLUTION

There is great variety of modes of sex determination in vertebrates, including chromosomal systems (XX:XY male heterogamety, ZW:ZZ female heterogamety), genetic and environmental sex determination. Although gonad differentiation seems to follow the same plan in reptiles, birds and mammals, an evolutionary relation between the sex chromosomes and sex determining genes of different vertebrates seems unlikely. There is no obvious genetic homology between the bird Z and the basic mammalian X (Graves 1995), implying that the XX:XY and ZW:ZZ systems evolved independently from different autosomal pairs in a primitive reptile. Not surprisingly, human *SRY* detects no sex-specific bands in birds or reptiles (Griffiths *et al.* 1991). Different genes must

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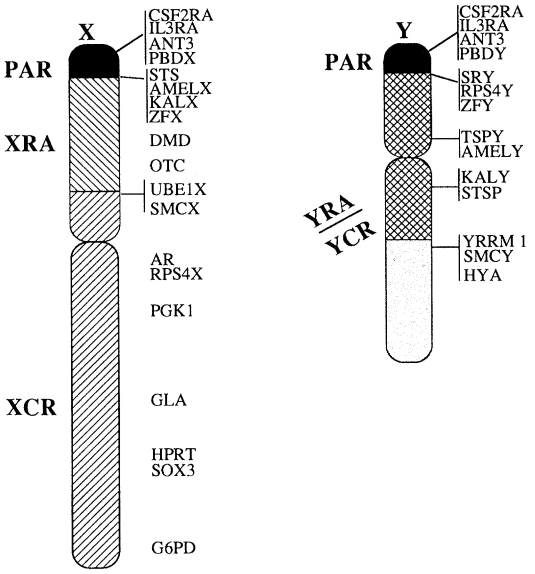

Figure 1. The evolutionary origin of regions of the human sex chromosomes. The X chromosome consists of a conserved region XCR (right hatch), shared by the X of all mammals, and a recently added region XRA (left hatch), which is on the X only in eutherians. The Y also consists of a conserved region YCR and a recently added region YRA, though these are scrambled (cross hatched). The X and Y pair only over part of the recently added regions (the pseudoautosomal region, or PAR, solid).

therefore act to control different steps of a common pathway in different vertebrates.

How did a homologous autosome pair become heteromorphic and acquire a sex determining function in mammals? In *Drosophila*, the X and Y are thought to have diverged by the gradual degradation of the Y after suppression of recombination to protect an association between two or more genes required in the male (Lucchesi 1978), and the same process accounts well for the array of dead and dying genes on the mammalian Y. The protection of the Y from recombination may be ultimately responsible for its degradation (Charlesworth 1990), either by stochastic elimination of Y chromosomes with the fewest mutants in small populations ('Muller's ratchet'), or the selection for and fixation of Y chromosomes which contain a favourable new variant, but also deleterious mutations in other genes (the 'hitchhiker hypothesis').

To deduce the genetic make-up and function of the original mammalian sex chromosomes, we have compared sex chromosomes and sex determining genes in the mammals most distantly related to eutherians. Marsupials (which diverged from eutherians about 130 Ma BP) and monotremes (which diverged even earlier from the therian line of descent) have heteromorphic X and Y chromosomes, but their size, pairing relations and gene contents differ from those of eutherian mammals in revealing ways. Marsupials have a smaller basic X (about 3% of the haploid complement), and a tiny Y, which do not appear to undergo homologous pairing and recombination (Sharp 1982). Monotremes have large X and Y chromosomes, which pair over an entire arm of the X and Y, and are part of a long translocation chain (Murtagh 1977).

Mapping studies in my laboratory over many years have shown that genes from the long arm and pericentric region of the human X also map to the marsupial and monotreme X (Graves & Watson 1991), identifying a conserved region. This X conserved region (XCR) is likely to represent the original mammalian X, which has remained intact for at least 170 Ma. In contrast, markers from the rest of the short arm of the human X are autosomal in both marsupials and monotremes, suggesting that a region (XRA) was recently added to the eutherian X, after the divergence of the marsupials, but before the major eutherian radiations. These comparative studies identify the evolutionary origins of different regions of the human X chromosome (see figure 1).

Of particular interest is the observation that several human X-Y shared genes lie in the XRA, implying that autosomal regions were added, not only to the eutherian X, but also to the Y. Because it is unlikely that the same region was independently added to the X and Y, the region is proposed to have been added initially to an ancient PAR of one partially differentiated sex chromosome, then recombined onto the other, enlarging the PAR. The Y chromosome, too, is composed of a conserved YCR and a recently added YRA, though these have been scrambled by rearrangements.

Based on this evidence, I propose that the sex chromosomes of present day placental mammals have evolved by cycles of autosomal addition to one partner, recombination onto the other, then progressive attrition of Y-borne sequences (figure 2). The original proto-X and -Y diverged as sequences within a non-recombining region on the YCR were mutated or lost, and the unpartnered genes in the XCR were recruited into the inactivation system. The autosomal region which was added to the X and Y (initially homologous, paired at meiosis, and not inactivated) became subject to the same forces of selection and drift which resulted in progressive degradation of the original Y. Genes within the YRA therefore became progressively mutated and lost, and their partners within the XRA became inactivated.

This cycle of addition and attrition must have occurred several times (figure 2) to explain why genes on the short arm of the human X map to at least three autosomal sites in marsupials and monotremes. The first rearrangement added autosomal region A1 to the X and Y, forming homologous added regions XRA1 and YRA1; the second added segments XRA2 and YRA2 etc. Each addition enlarged the PAR, then rearrangement, inactivation and deletion of the Y progressively differentiated each YRA from its XRA. The X has therefore been enlarged in stages, whereas the Y has gone through cycles of incremental enlargement and gradual attrition. Internal rearrangements, such as the inversion which occurred recently in the human-chimpanzee Y lineage (Schempp *et al.* 1993), would have progressively scrambled the genes in these added blocks, particularly in the Y, which is prone to rearrangement. The addition-attrition hy-

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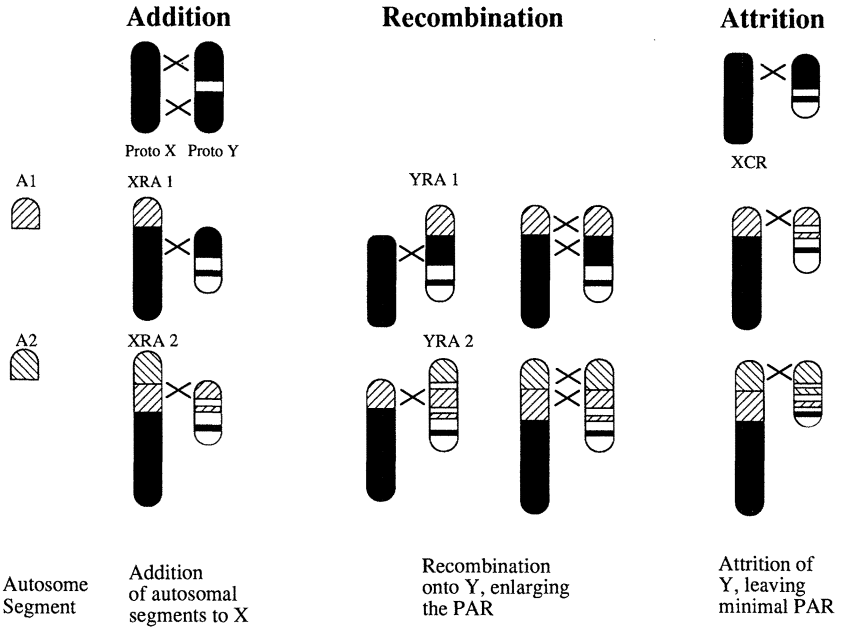

Figure 2. The addition-attrition hypothesis for the origin of mammalian sex chromosomes. The original sex chromosomes (solid) were homologous except at a sex determining locus (white) and underwent homologous pairing (represented by crosses). The Y was progressively degraded, leaving few functional genes (solid stripes on a white background of non-coding DNA), and a small paired region (PAR) at the terminus. Addition of an autosomal segment A1 to the X (XRA1) was followed by recombination within the PAR, adding the segment to the Y (YRA1) and producing enlarged sex chromosomes paired over the added region. The enlarged Y was further degraded, leaving a few functional genes (hatched stripe) and a new PAR. The cycle of addition and attrition was repeated with autosomal region A2.

pothesis predicts that different additions may have occurred in different lineages, so that sex chromosomes of other mammals may be found to contain blocks of genes which are autosomal in humans. Indeed, this must be true of monotreme sex chromosomes, which are larger than human sex chromosomes, but share only part of their gene complement.

The hypothesis also predicts that the PAR is merely a relic of the last addition to X and Y. PARs with different gene contents could be generated in different mammal groups by different recent additions (see figure 2), or by internal rearrangements producing different terminal regions, which are the last to be differentiated. In fact, there is little evidence for the conservation of the PAR between eutherian species.

4. ACQUISITION OF MALE-SPECIFIC FUNCTIONS BY Y-BORNE GENES, AND THE EVOLUTION OF *SRY*.

The addition-attrition hypothesis proposes that genes were added to the Y chromosome, isolated from recombination, then subjected to mutation and loss. Most of the thousands of genes on the X have been inactivated and eliminated from the corresponding regions of the Y; the dozen or so survivors have remained, either because they have been added recently and have not yet had time to be degraded and lost, or because they have taken on a unique male-specific function that ensure their survival. Thus it would seem that genes on the Y are either selectable or disposable. *SRY*, *ZFY* and *Ube1y* evidently fall into the selectable category (as judged by their presence on the Y in distantly related species), at least until their function is taken over by some other gene, as has evidently happened to *UBE1Y* in the primate lineage. *STS*, *KAL*, *AMEL*, *PBD* and all the pseudoautosomal genes fall into the disposable category, and will ultimately follow the thousands of other Y-borne genes into evolutionary oblivion.

Comparisons of expression patterns and sequences between and within species support the view that *ZFY*, *UBE1Y* and *SRY* each evolved from its X-borne partner *ZFX*, *UBE1X* and *SOX3* (figure 3). The ubiquitous expression pattern of mouse *Zfx* and *Ube1x* implies important general roles in both sexes, whereas expression of the Y-borne partner is limited to the gonad (Koopman *et al.* 1990; Mitchell *et al.* 1991). Similarly, *Sox*3 is expressed more widely than *Sry*. Each X linked gene is highly conserved between species, whereas its Y-borne partner has undergone considerable sequence divergence, and in some cases, amplification or loss (Pamilo & Bianchi 1993, Mitchell *et al.* 1991). For instance, *SOX*3 specifies almost identical products in human, mouse and marsupials, whereas *SRY* has changed rapidly in rodent and primate evolution, and is almost unrecognizable in more distantly related mammals (Tucker & Lundrigan 1993; Whitfield *et al.* 1993; Foster *et al.* 1992). *SRY* is even amplified in some rodents (Nagamine & Carlisle 1994).

Thus is seems likely that the evolution of SRY has followed the same haphazard course as has the evolution of other genes on the Y. Each Y-borne gene

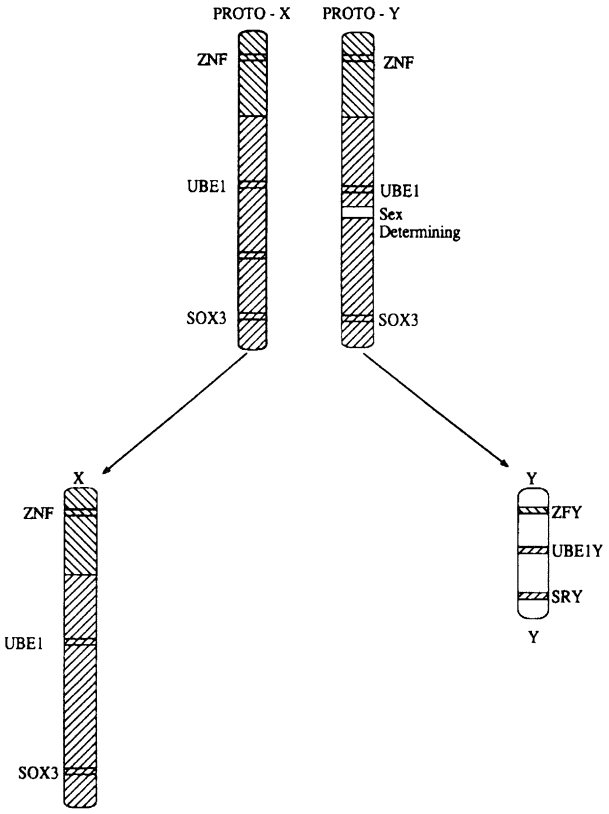

Figure 3. Evolution of genes on the Y with functions in sex determination and differentiation. As the Y was progressively degraded, genes *SOX*3 and *UBE1* on the original proto-X and Y (right hatch) diverged, acquiring male-specific functions as *SRY* and *UBE1Y*. Later, an autosomal region containing the zinc finger gene *ZNF* was added as part of the XRA (left hatch), and diverged to produce *ZFY* with a male-specific function.

evolved from an X linked gene with a general function in both sexes, but as the Y differentiated from the X, the Y-borne allele acquired selectable male-specific functions which protected it from inactivation and deletion.

5. CONCLUSIONS

The relatively recent evolution of the sex-determining role of *SRY* may account for the unexpected intricacies of mammalian sex determining pathways. The dominant action of the testis determining factor on the Y chromosome initially seduced us into believing that 'sex is simple', and testis determination involves a linear series of steps, operated by TDF acting as a 'master switch' to activate downstream events. The reality may be far more complex, even bizarre, with *SRY* acting, not directly as a transcriptional activator, but as part of a long and tortuous control pathway which may have been recruited from quite a different function (Graves 1995).

The obvious importance of reproduction in evolution and the morphological similarities in gonadogenesis, has led to the expectation that sex determining pathways should be highly conserved among vertebrates. However, although the gonadogenesis pathway itself may be very conserved in mammals and other vertebrates, the genes that control it have evolved rapidly. That they are still evolving is apparent in variant sex determining systems (Fredga 1988), such as in the mole vole, which lacks a Y chromosome and has no *SRY* (Just *et al.* 1995).

The apparent simplicity of mammalian sex determining pathways may just reflect our lack of knowledge, and the labyrinthine pathways of genetic interactions that control sex determination in *Drosophila* and *Caenorhabditis* might warn us that mammalian sex is unlikely to be any more straightforward. Sex determining genes in mammals, as in *Drosophila*, may act very indirectly, and may even retain functions other than sex determination. These complexities are hard to understand in terms of efficient function, but they make excellent sense in terms of the evolution of the mammalian Y chromosome.

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REFERENCES

Adler, D. A., Bressler, S. L., Chapman, V. M., Page, D. C & Disteche, C. M. 1991 Inactivation of the *Zfx* gene on the mouse X chromosome. *Proc. natn. Acad. Sci. U.S.A.* **88**, 4592–4595.

Agulnik, A. I., Mitchell, M. J., Mattei, M-G. *et al.* 1994*a* A novel X gene with a widely transcribed Y-linked homologue escapes X-inactivation in mouse and human. *Hum. molec. Genet.* **3**, 879–884.

Agulnik, A. I., Mitchell, M. J., Lerner, J. L., Woods, D. R. & Bishop, C. E. 1994*b* A mouse Y chromosome gene encoded by a region essential for spermatogenesis and expression of male-specific minor histocompatibility antigens. *Hum. molec. Genet.* **3**, 873–878.

Ashworth, A., Rastan, S., Lovell-Badge, B. R. & Kay, G. 1991 X-chromosome inactivation may explain the difference in viability of XO humans and mice. *Nature, Lond.* **351**, 40640–40648.

Burgoyne, P. 1993 Deletion mapping the functions of the mouse Y chromosome. In *Sex chromosomes and sex determining genes* (ed. K. C. Reed & J. A. M. Graves), pp 353–368. Chur Swizerland: Harwood Academic.

Charlesworth, B. 1991 The evolution of sex chromosomes. *Science, Wash.* **251**, 1030–1033.

Clepet, C., Schafer, A. J., Sinclair, A. H., Palmer, M.S., Lovell-Badge, R. & Goodfellow, P. N. 1993 The human *SRY* transcript. *Hum. molec. Genet.* **2**, 2007–2012.

Collignon, J. & Lovell-Badge, R. 1993 *SRY*-related genes *SOX-1* and *SOX-2* and inductive interactions in the nervous system. *Development* (In the press.)

Eicher, E. M. 1988 Autosomal genes involved in mammalian primary sex determination. *Phil. Trans. R. Soc. Lond.* B **322**, 109–118.

Ferrari S., Harley, S., Pontiggia, V. R., Goodfellow, P. N., Lovell-Badge, R. & Bianchi, M. E. 1992 *SRY*, like HMG1, recognises sharp angles in DNA. *EMBO J.* **11**, 4497–4506.

Foster, J. W., Brennan, F. E., Hampikian, G. K. *et al.* 1992 Evolution of sex determination and the Y chromosome: *SRY*-related sequences in marsupials. *Nature, Lond.* **359**, 531–533.

Foster, J. W. & Graves, J. A. M. 1994 An *SRY*-related sequence on the marsupial X chromosome: implications

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

for the evolution of the mammalian testis-determining gene. *Proc. natn. Acad. Sci. U.S.A.* **91**, 1927–1931.
Foster, J. W., Dominguez-Steglich, M., Guioli, S. *et al.* 1994 Campomelic dysplasia and autosomal sex reversal caused by mutations in an *SRY*-related gene. *Nature, Lond.* **372**, 525–530.
Fredga, K. 1988 Aberrant chromosomal sex-determining mechanisms in mammals, with special reference to species with XY females. *Phil. Trans. R. Soc. Lond.* B **322**, 83–95.
Graves, J. A. M. 1995 The origin and function of the mammalian Y chromosome and Y-borne genes – an evolving understanding. *BioEssays* **17**, 311–320.
Graves, J. A. M. & Watson, J. M. 1991 Mammalian sex chromosomes: evolution of organization and function. *Chromosoma* **101**, 63–68.
Graves, J. A M. & Schmidt, M. M. 1992 Mammalian sex chromosomes: design or accident? *Curr. Opin. Genet. Dev.* **2**, 890–901.
Griffiths, R. 1991 The isolation of conserved DNA sequences related to the human sex-determining region Y gene from the lesser black-backed gull (*Larus fuscus*). *Proc. R. Soc. Lond.* B. **224**, 123–128.
Gubbay, J., Collignon, J., Koopman P. *et al.* 1990 A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. *Nature, Lond.* **346**, 245–250.
Harley, V. R., Jackson, D. I., Hextall, P. J. 1992 DNA binding activity of recombinant *SRY* from normal males and XY females. *Science, Wash.* **255**, 453–456.
Hawkins, J. R., Taylor, A., Berta, P., Levilliers, J., Van der Auwera, B. & Goodfellow, P. N. 1992 Mutational analysis of *SRY*: Nonsense and missense mutations in XY sex reversal. *Hum. Genet.* **88**, 471–474.
Koopman, P., Munsterberg, A., Capel, B., Vivian, N. & Lovell-Badge, B. R. 1990 Expression of a candidate sex-determining gene during mouse testis differentiation. *Nature, Lond.* **348**, 450–452.
Koopman, P., Gubbay, J., Vivian, N., Goodfellow, P. & Lovell-Badge, B. R. 1991 Male development of chromosomally female mice transgenic for *SRY*. *Nature, Lond.* **351**, 117–121.
Lucchesi, J. C. 1978 Gene dosage compensation and the evolution of sex chromosomes. *Science, Wash.* **202**, 711–716.
Ma, K., Inglis, J. D., Sharkey, A. *et al.* 1993 A Y chromosome gene family with RNA-binding protein homology: candidates for the azoospermia factor *AZF* controlling human spermatogenesis. *Cell* **75**, 1287–1295.
Manz, E., Schneiders, F., Brechlin, A. M. & Schmidtke, J. 1993 TSPY-related sequences represent a microheterogeneous gene family organized as constitutive elements in DYZ5 tandem repeat units on the human Y chromosome. *Genomics* **17**, 726–731.
McElreavey, K. E., Vilain, E., Abbas, N., Herskowitz, I. & Fellous, M. 1993 A regulatory cascade hypothesis for mammalian sex determination: SRY represses a negative regulator of male development. *Proc. natn. Acad. Sci. U.S.A.* **90**, 3368–3372.
Mitchell, M. J., Woods, D. R., Tucker, P. K., Opp, J. S. & Bishop, C. E. 1991 Homology of a candidate spermatogenic gene from the mouse Y chromosome to the ubiquitin-activating enzyme E1. *Nature, Lond.* **354**, 483–486.
Murtagh, C. E. 1977 A unique cytogenetic system in monotremes. *Chromosoma* **65**, 37–57.
Nagamine, C. M. & Carlisle, C. 1994 Duplication and amplification of the *SRY* locus in Muridae. *First Y Chromosome Workshop* (*abstr.* 24).
Pamilo, P. & Bianchi, N. O. 1993 Evolution of the *Zfx* and *Zfy* genes: Rates and interdependence between the genes. *Molec. Biol. Evol.* **10**, 271–281.
Schempp, W. & Toder, R. 1993 Molecular cytogenetic studies on the evolution of sex chromosomes in primates. In *Sex chromosomes and sex determining genes* (ed. K. C. Reed & J. A. M. Graves), pp 137–152. Chur Swizerland: Harwood Academic.
Schneider-Gadicke, G. A., Beer, R. P., Brown, L. G., Nussbaum, R. & Page, D. C. 1989 ZFX has a gene structure similar to ZFY, the putative human sex determinant, and escapes X inactivation. *Cell* **57**, 1247–1258.
Sharp, P. 1982 Sex chromosome pairing during male meiosis in marsupials. *Chromosoma* **86**, 27–47.
Sinclair, A. H., Berta, P., Palmer, M. S. *et al.* 1990 A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature, Lond.* **346**, 240–244.
Tucker, P. K. & Lundrigan, B. L. 1993 Rapid evolution of the sex determining locus in old world mice and rats. *Nature, Lond.* **364**, 715–717.
Whitfield, L. S., Lovell-Badge, R. & Goodfellow, P. N. 1993 Rapid sequence evolution of the mammalian sex-determining gene *SRY*. *Nature, Lond.* **364**, 713–715.
Wright, E., Hargrave, R., Christiansen, J. *et al.* 1995 The *SRY*-related gene *SOX*9 is expressed during chondrogenesis in mouse embryos. *Nature Genet.* **9**, 15–20.
Zanaria, E., Muscatelli, F., Bardoni, B. *et al.* 1994 An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. *Nature, Lond.* **372**, 635–641.

Discussion

M. A. Ferguson-Smith (*Department of Pathology, University of Cambridge, U.K.*). A number of genes in the differential part of the human Y chromosome are known to be expressed and to have copies on the X chromosome which escape inactivation. A double dose of the genes is important for the phenotype as, for example, haplo-insufficiency is associated with Turner's syndrome. Do you think that the presence of these loci helps to maintain the integrity of the Y against the voluntary pressure towards Y attrition?

J. A. M. Graves. I don't doubt that selection for retention of a double dose of some X–Y shared genes occurs. Although dosage inequities between male and female are cancelled out by loss from the Y and incorporation into the X inactivation system, there may be genes whose dose proportional to that of an autosomal gene is critical. However, I doubt whether this selection exerts more than a holding action, since in mouse, most X–Y shared genes are no longer exempt from inactivation, and XO females are viable and fertile.

A. McLaren (*Wellcome/CRC Institute, Cambridge, U.K.*). Many marsupial Y chromosomes are tiny. What is known about the pairing between marsupial X+Y chromosomes at meiosis? Do the tiny Y chromosomes often get lost, giving rise to XO embryos?

J. A. M. Graves. Peter Sharpe found that the marsupial X and Y chromosomes do attach end-to-end (sometimes both ends) at diakineses, but there appears to be no synaptinemal complex and no chiasmata are visible. Only a few XO and XXY marsupials have been reported, so sex chromosome non-disjunction doesn't appear to be very frequent. The Y is often lost in culture. In some species of bandicoots, the Y chromosome gets lost from somatic tissues – but so does the inactive X, so its loss probably has more to do with the Y

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chromosome's uselessness than its size! The marsupial X and Y seem to have run out of pseudoautosomal region, and we might expect the poor little Y to disappear entirely in the next 100 million years. In some marsupials species, the Y has acquired lumps of heterochromatin, and translocations with autosomes are quite common; perhaps this addition of ballast to the Y will stave off its demise.

U. Mittwoch (*Department of Anatomy, Queen Mary & Westfield College, London*). Is it not likely that different mammals experience different problems during the process of sex determination and therefore require differences in the genes that bring it about? For instance, in eutherian mammals, virtually the entire process of male sex differentiation takes place within the placenta, in an environment of rapidly rising oestrogen levels, whereas in marsupials the major part of male sexual development occurs after birth. This suggests that eutherian males need to develop their sexuality faster than marsupials, and the underlying genetic basis might be expected to reflect this.

J. A. M. Graves. It's true that the marsupial embryo can escape the mother's hormonal influences before it has to determine testis; also administration of oestrogen to male pouch young up to a few days after birth causes gonadal sex reversal. It is an interesting thought that eutherians might have evolved additional genetic mechanisms to overcome this problem. There is no evidence that eutherian embryos determine testis any faster than marsupials (I would have thought the reverse), but I suppose it is possible that eutherians have evolved other genes to cope with making a testis 'in a sea of maternal estrogen'. The decision to make scrotum or mammary glands is taken independently before birth when estrogen is already present, as Professor Renfree points out (see her comment).

M. B. Renfree (*University of Melbourne, Parkville, Melbourne Vic. 3052, Australia*). As a comment in response to Ursula Mittwoch's suggestion that marsupials have to give birth early and do their sex differentiation later to avoid being 'bathed in a sea of oestrogen'. My comment is that marsupials do have oestrogen in the mother before birth, and in the swamp wallaby (*Wallabia bicolor*) females come into oestrus one day before birth, and yet have normal male offspring. Marsupials also have a fully functional placenta, and in both the tammar and quokka the placenta has some steroidogenic activity.

P. Burgoyne (*NIMR, Mill Hill, London*). I am fairly certain that it was Roger Short who suggested many years ago that the mammalian Y chromosomal sex-determining system had evolved in order to overcome the problems associated with foetal development occuring in a sea of estrogens. In lower vertebrates oestrogens cause male to female sex reversal, so Roger Short envisaged that mammalian testis determination required a genetic control which acted early to lock the gonad into the pathway of testis differentiation before the gonad reached the stage of responsiveness to estrogen. If this were true, then an egg-laying mammal such as the Platypus would be at a point in the evolution of mammals when the Y determinant (SRY) is not yet required, which would be consistent with your failure to identify a Platypus *Sry*.

J. A. M. Graves. In monotremes testis determination occurs in an egg, removed from maternal influences. Nobody has tested whether oestrogen administration causes sex reversal, but I will bet it does. However, I don't think this is the driving force behind evolving a Y chromosome or SRY in mammals. After all, birds and many oviparous reptiles have highly differentiated sex chromosomes, and marsupials have a Y which bears SRY.

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