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Review

Mammalian sex—Origin and evolution of the Y chromosome and *SRY*

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Abstract

Sex determination in vertebrates is accomplished through a highly conserved genetic pathway. But surprisingly, the downstream events may be activated by a variety of triggers, including sex determining genes and environmental cues. Amongst species with genetic sex determination, the sex determining gene is anything but conserved, and the chromosomes that bear this master switch subscribe to special rules of evolution and function. In mammals, with a few notable exceptions, female are homogametic (XX) and males have a single X and a small, heterochromatic and gene poor Y that bears a male dominant sex determining gene *SRY*. The bird sex chromosome system is the converse in that females are the heterogametic sex (ZW) and males the homogametic sex (ZZ). There is no *SRY* in birds, and the dosage-sensitive Z-borne *DMRT1* gene is a credible candidate sex determining gene. Different sex determining switches seem therefore to have evolved independently in different lineages, although the complex sex chromosomes of the platypus offer us tantalizing clues that the mammal XY system may have evolved directly from an ancient reptile ZW system. In this review we will discuss the organization and evolution of the sex chromosomes across a broad range of mammals, and speculate on how the Y chromosome, and *SRY*, evolved.

Keywords: *SRY*; Y chromosome; X chromosome; Comparative genomics; Placental mammals; Marsupials

Contents

1. Introduction

Sex determination in vertebrates – the development of an embryo as a male or a female – seems to be controlled by a trigger that switches development of the embryonic gonad into a testis or an ovary developmental pathway. This gene trigger, and the chromosome that bears it, has been the subject of intense investigation in mammals, and the male-specific Y chromosome and the Y-borne male dominant *SRY* gene have been well characterized, at least in humans and mice.

We might expect that sex chromosomes and sex determination, being critical for the survival of a species, are conserved in

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other vertebrate groups. This is far from the case, for amongst different vertebrates, the sex determining gene and even the identity of the sex chromosomes may be quite different. Many reptiles and some fish lack sex chromosomes, and rely on environmental cues – commonly the temperature at which eggs are incubated – to control male or female development. However, once sex is determined, both environmental sex determination (ESD) and different systems of genetic sex determination (GSD) all seem to operate through a down stream genetic pathway that is highly conserved in all vertebrates.

How sex chromosomes evolved has been debated for decades after H.J. Muller's suggestion that they differentiated from a pair of ordinary autosomes after one acquired a sex determining gene. Ohno [1] suggested that the Z and W sex chromosomes of different snake families reflected a gradual degradation of a female-specific W chromosome, and the mammalian X and Y were proposed to have originated in a parallel manner [2].

Here we review the characteristics of mammal sex chromosomes, explore their relationship to other sex chromosome systems, and discuss the trajectory through which the mammal Y differentiated and the *SRY* gene evolved. Starting with the well characterized human X and Y pair, we compare the sex chromosomes of closely and distantly related mammals, then explore how the evolution of the Y from an ancestral autosome relates to the evolution of the *SRY* gene from an autosomal gene expressed in brain and testis.

2. Mammalian sex chromosomes

2.1. Human sex chromosomes

The human X and Y chromosomes are highly differentiated. The 155 Mb X chromosome represents about 5% of the haploid genome and bears ∼1100 genes with a mix of housekeeping and specialist functions [3]. The mammal X is highly conserved between species, to the extent that gene order and content is almost identical between species [4] with the exception of the murid rodent X, in which gene order is scrambled [5]. Although most genes on the human X chromosome are not involved with sex, there is an increased frequency (relative to autosomes) of sex and reproduction related (SRR) genes [6]. Interestingly, many SSR genes on the human X are also involved in X-linked mental retardation (XLMR) syndromes, and it was suggested that the same genes were recruited for function in the brain, testis and placenta because they were responsible for human speciation [7], or because different selective forces acted independently on the same multifunctional proteins [8].

The human Y chromosome is much smaller, and rich in repetitive sequence that endow it with special staining characteristics (heterochromatin). Although it looks completely different from the X chromosome, it shares a small region of homology with the X (the pseudoautosomal region, PAR), within which there is an obligatory recombination event during male meiosis that mediates X and Y segregation. A second small PAR lies at the other end of the human X and Y. The rest of the Y is male-specific: this MSY represents about 2% of the haploid genome (∼60 Mb). Much of the MSY is composed of simple-sequence repetitive DNA, and contains no genes. Even the euchromatic 24 Mb [9] contains few active genes; of 172 transcriptional units on the MSY, many are untranslatable pseudogenes and others are amplification products. The MSY codes for only 27 distinct proteins [9]. Of these 27 protein coding genes, 20 have a partner on the X chromosome.

X–Y homology in the PAR and the preponderance of XY shared genes support the proposal that the mammalian X and Y originated from an autosomal pair. Differentiation of the proto-Y began when it acquired a male determining locus, there was then accumulation of other male-advantage genes in a region across which recombination was suppressed [1,2]. Lack of recombination resulted in progressive degradation because selection no longer acted upon a single gene, but rather the entire MSY. Under these conditions the Y degraded because of higher variation, drift and inefficient selection. Mutation is higher in the testis than the ovary because of the additional division cycles and the repetitive structure. Selection is inefficient against a deleterious allele on an otherwise good Y, or for a favourable allele on a bad Y, and genetic drift becomes important because of the inability to recombine un-mutated regions of the Y (reviewed in [10]). Of the ∼1100 genes on the ancestral Y (now represented by the X chromosome) only a total of 45 survive. Being confined to males, many of these evolved a function in male reproduction, and came under positive selection.

The deletion and inactivation of most of the Y led to problems of chromosome segregation and gene dosage. Chromosome pairing at meiosis was compromised, such that special mechanisms that ensure high recombination in the tiny PAR evolved. With loss of gene function on the Y came gene dosage imbalance between the sexes that is compensated for by upregulation of the now unpartnered genes on the X [11]. To compensate for this, transcriptional silencing of one X chromosome in the somatic cells of females evolved. X chromosome inactivation (XCI) is a complex epigenetic mechanism controlled by the *XIST* gene.

2.2. Sex chromosomes of other placental mammals

All placental mammals have an XX female XY male sex determining system or some simple variant of it, and gene mapping and chromosome painting show that the X chromosome is almost identical, even between the most distantly related species. However, the Y chromosome differs morphologically and genetically between species.

There are three groups of extant mammals. Placental mammals (mammalian Infraclass Eutheria) diverged from marsupials (Metatheria) about 180 million years ago (MYA), and Subclass Theria (containing placentals and marsupials) diverged from the egg-laying monotremes (Subclass Prototheria, containing the platypus and echidna) about 210 MYA (Fig. 1). Placental mammals are now divided into four superordinal clades; Euarchontoglires (or Supraprimates), Laurasiatheria, Xenarthra and Afrotheria [12,13]). Supraprimates diverged from Afrotheria about 105 MYA, Xenarthra about 100 MYA and Laurasiatheria about 90 MYA [14].

There is a huge amount of data available about the human X and Y, as well as for the X and Y of our model species (mouse),

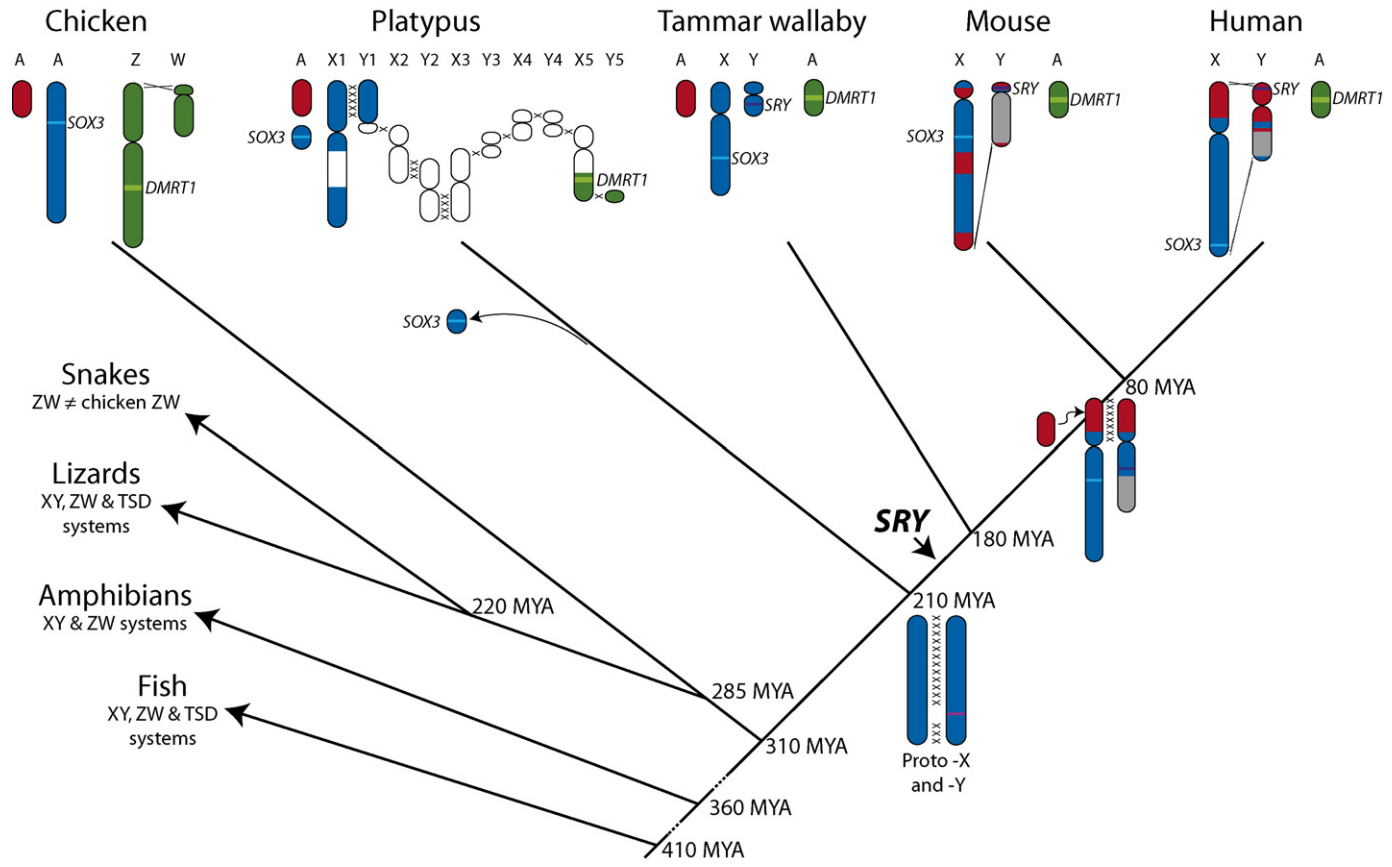

Fig. 1. Overview of sex chromosome evolution in vertebrates. Homology between genomes of representative species are indicated by different colours and *SRY*/*SOX3* and *DMRT1* are tracked in each species. Homologues of the reptile/bird ZW pair are indicated in green, homologues of the ancient X chromosome shared by marsupials and placentals (XCR/YCR) are blue and homologues of the autosome that was added to the X and Y (XAR/YAR) are red. Grey regions of the Y chromosomes represents heterochromatin. Major events in sex chromosome evolution are marked on the tree. An arrow indicates the birth of *SRY*. The mammalian proto-X and -Y, addition to the placental sex chromosomes and loss from the monotreme sex chromosomes are indicated on the tree. Different sex determination systems represented in the snakes, lizards, amphibians and fish are indicated. These systems are female heterogamety (ZW), male heterogamety (XY) and temperature dependent sex determination (TSD).

which belongs to the same superordinal clade (Supraprimates). Much less is known of the Laurasiatheria, which includes economically important species such as cow, pig and horse, as well as carnivores such as cat and dog. Almost nothing is known about the gene content, organization and inactivation of the sex chromosomes in other mammalian groups, especially the basal South American xenarthrans and African afrotherians. Oddly enough, rodents are rank outsiders in many comparisons of closely and distantly related placentals, although they have recently been placed more closely to humans [13].

The X chromosome is highly conserved in size (∼5% of the haploid genome) and gene content between all placentals, as first observed by Ohno [1] and referred to as Ohno's Law. Gene mapping in Laurasiatheria has revealed that the order of genes on the X chromosome is almost identical in several species [4,15–17]. Although gene order is unknown in afrotherian and xenarthran X chromosomes, complete homology to the human X chromosome has been established by chromosome painting [18,19]. However, the mouse X appears to have been rearranged compared to the X of other mammals, although is genetically almost identical [20].

Inactivation of a single X chromosome in females seems to be a ubiquitous feature of placental mammals. Humans, mice, and even afrotherians and xenarthrans share several classic features of XCI such as sex chromatin formation, asynchronous replication, an *XIST* locus and accumulation of LINEs [21,22]. However, the details of the molecular mechanism are significantly different between humans and mice, the only two mammals in which intensive studies have yet been done.

The Y chromosome is much more variable than the X, differing between species in size and gene content, and in homology relationships to the X. A single PAR is shared between the X and Y of mouse, cow and horse and appears to be critical for fertility. There is recent evidence that afrotherian sex chromosomes also have a PAR, represented by a small region of synaptonemal complex protein 1 (SCP1) between the X and Y during male meiosis in the Cape elephant shrew (Paul D. Waters, in preparation). Y chromosomes of different species show considerable variation in heterochromatin content, from the small sheep Y chromosome, to the largely heterochromatic elephant Y [23]. Repetitive sequences are very poorly conserved, and a Y chromosome paint prepared from one species usually will not hybridize to

the Y of even quite closely related species (e.g. between sheep and cow).

The gene content of mammalian Y chromosomes has been defined only recently. Once thought to code for traits such as hairy ears and scaly skin, the human Y chromosome was considered to be devoid of genes when no families could be found displaying genuine Y-linked inheritance [24]. However, in 1959, observations of human and mouse XO females and XXY males [25–27] were interpreted to mean that a male determining factor was on the Y chromosome. More recently several functions have been attributed to the Y, including factors involved in sperm production and stature.

Other than human, chimpanzee and mouse, the gene content of Y chromosomes is poorly known, even for species such as dog and cow whose (female) genomes have been sequenced at depth. There is considerable variation of Y gene content between species, although they overlap, and all contain *SRY* plus several genes known to be required for spermatogenesis. As for human, most genes on the Y in other species have paralogues on the X, and comparison of genes on the Y in human, mouse, cow, horse with genes on the X chromosome suggests that different subsets of genes from an ancient autosome were retained in some species and lost in others (Fig. 2). Expression patterns of orthologous genes in different species are not necessarily the same; for instance, human *ZFY* is a ubiquitously expressed housekeeping gene, whereas mouse *ZFY* is testis-specific and required for spermatogenesis. This suggests that Y gene function as well as gene content may have changed during evolution in some lineages.

2.3. Marsupial sex chromosomes

Marsupial sex chromosomes are generally smaller than placental sex chromosomes: the basic X chromosome represents about 3% of the haploid genome and the Y chromosome is tiny. However, the marsupial X chromosome shares many genes with the long arm and pericentric region of the human X ([28] and listed in [29]), implying that therian sex chromosomes are monophyletic. However, genes on the short arm of the human X distal to Xp11.23 are located on autosomes in marsupials [29,30] and also monotremes [31]. This defined an ancient X conserved region (XCR) and a region (XAR) added to the eutherian X [32]. Comparative mapping of human X-borne genes in chicken confirms that these two regions were separate in a common ancestor of birds and mammals 310 MYA, and that marsupials have retained the ancestral arrangement of genes [33] (Fig. 1).

The marsupial Y chromosome should be an ideal candidate for full characterization because it is tiny and has little heterochromatin [34]. However, sequencing of the female genomes of the opossum (*Monodelphis domestica*) and a model kangaroo (the tammar wallaby, *Macropus eugenii*), provide no information on the Y. Our knowledge of the marsupial Y therefore depends on comparative gene mapping, and on analysis of Y-enriched BAC libraries [35].

Because most human MSY protein coding units evolved from X homologues, it was not surprising to find that the Y, too, is composed of conserved (YCR) and added regions (YAR) [36]. Of the 20 distinct protein coding units on the human MSY that have a partner on the X, only five are present on the marsupial Y (YCR) and 13 are autosomal (YAR). However, the marsupial Y contains several novel genes with no orthologues on the Y of any placental mammal. The observation that they have paralogues on the human X [37,38] confirms our conclusion that different mammal lineages all started off with the same proto-Y, which was equivalent to the X, but have lost different subsets of genes [8] (Fig. 2).

The five genes conserved between placentals and marsupials, which include *SRY* (sex determination) and *RBMY* (candidate spermatogenesis factor), are likely to have been retained because they have selectable male functions. The four marsupial Y genes that appear to have been lost from the placental Y may have evolved a male-specific function in the marsupial lineage, or may have been lost from placentals when their function was replaced by another gene.

The loss of nearly all the 1100 original genes on the human Y over the 310 MY since humans and birds last shared a common ancestor means that genes have been lost from the Y at a rate of about 4 per million years. At this rate, the last 45 genes will be lost and the Y will disappear in about 12 million years, although positive selection of genes with an important male-specific functions may stave off the eventual disappearance of the Y [8]. However, even the possession of sex and spermatogenesis genes has not saved the Y in two groups of rodents; the mole voles of eastern Europe and the country rat of Japan [39,40], which lack a Y and have no *SRY* gene.

As with placental mammals, the loss of Y gene function imposes a need for some form of marsupial dosage compensation. Marsupials also undergo XCI, although it is different in several phenotypic and molecular respects [41], and is evidently not controlled by an *XIST* gene ([21], Hore et al., submitted).

2.4. Monotreme sex chromosomes

Monotremes have a complicated sex chromosome system that has provided new and startling information about the origin of the mammal XX:XY system. In platypus, females have five pairs of X chromosomes (X_1X_1 X_2X_2 X_3X_3 X_4X_4 X_5X_5) and males have five Xs and five Ys (X_1Y_1 X_2Y_2 X_3Y_3 X_4Y_4 X_5Y_5) that form a chain at meiosis [42,43]. The X_1 chromosome that lies at one end of the platypus translocation chain shares several genes with the human and marsupial X chromosomes [28] but has lost a region, probably from an ancestral PAR, that includes *RBMX* and genes that flank the *XIST* gene in placentals. This suggests that these genes gained their specialized functions only in the therian lineage after the monotreme-therian divergence 210 MYA [44].

Of particular interest is the platypus X_5 chromosome that lies at the far end of the translocation chain. This chromosome proved to contain an orthologue of the chicken *DMRT1* gene, the putative bird sex determining gene. The five platypus X chromosome represent ~15% of the genome and, although *DMRT1* might be present on X_5 by chance, it is likely there as a result of the chicken Z being a major constituent of an ancestral meiotic chain. This suggests an evolutionary link between the bird ZW system and the therian mammal XY system [42]. It has been

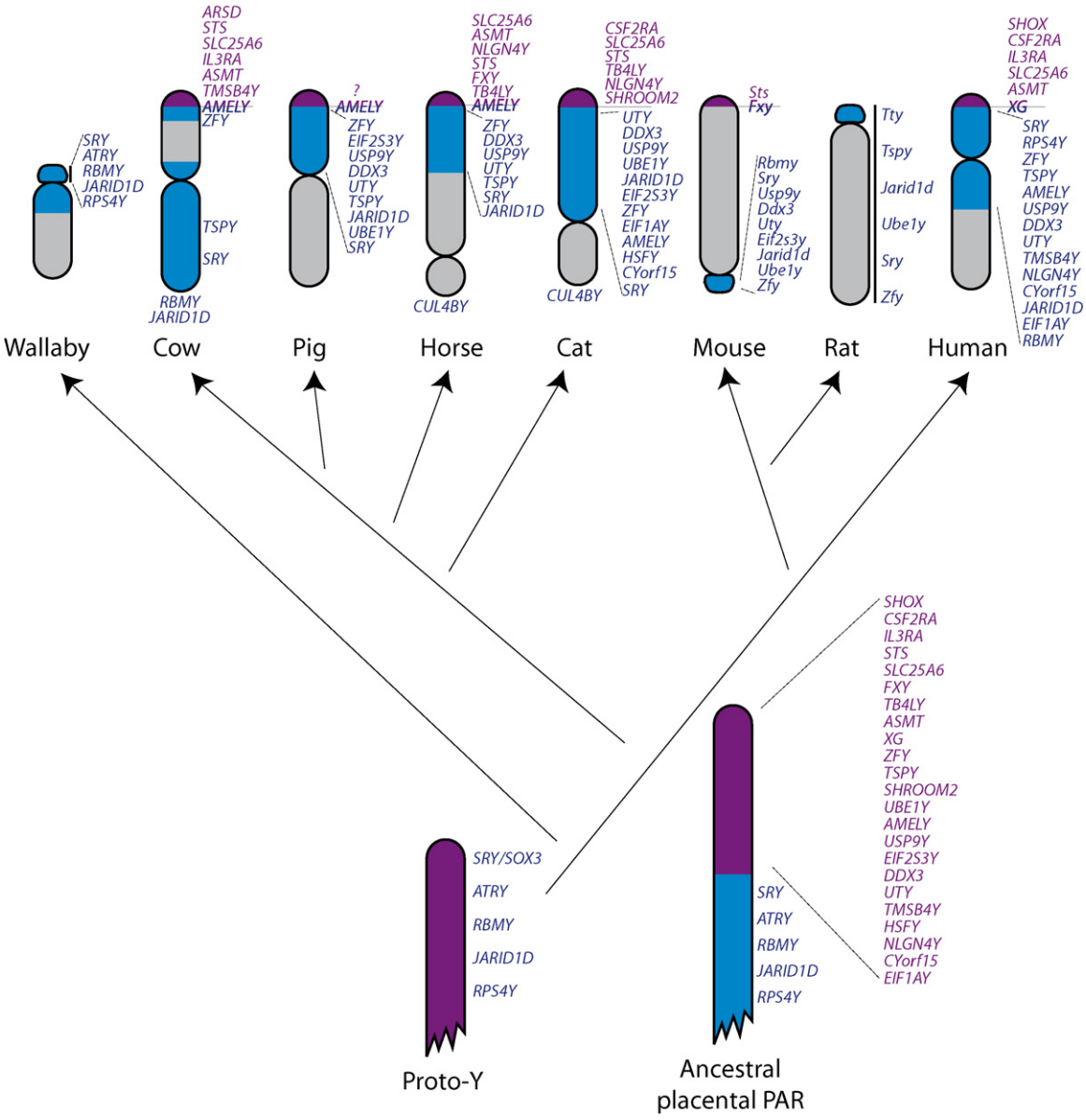

Fig. 2. Gene homology between extant mammalian Y chromosomes compared to ancestral mammalian Ys. Purple indicates pseudoautosomal regions, blue are euchromatic non-recombining regions of the Y and grey are heterochromatin. Genes span the pseudoautsomal boundary in cow, pig, horse, mouse and human. Gene orders on the rat and wallaby Y chromosomes are unknown, and genes listed under the chromosomes are those located on the Y but for which no positional data is available. Genes in the pig PAR are yet to be identified. The position and gene content of the rat PAR is unknown, but meiotic studies indicated that there is a PAR, which does not include *Sts*. Y chromosomes from different species contain different subsets of genes derived from the ancient proto-X and -Y pair.

suggested that the chain started with a translocation between an autosome and a bird-like ZW pair in an ancestral reptile-like mammal, and that this autosome assumed control of sex determination in placentals [45].

A major hurdle when interpreting the origin of the monotreme meiotic chain is determining the ancestral sauropsida (bird/reptile) and amniote (sauropsida/mammal) sex chromosome state. Recently, snakes were shown to have a ZW system that is conserved across serpents, but which is not equivalent to the bird ZW [46]. However, to determine the ancestral sex chromosome states in the groups outlined above, ZW systems of representatives from both lizards and turtles need to be investigated.

DMRT1 lies on the chicken Z chromosome, but not the W [47], and is expressed during gonadogenesis [48]. It is therefore present in a double dose in male and a single dose in female birds, making it a strong candidate for a dosage dependent sex-determining locus in birds. Consistent with this is the observation that in the emu *DMRT1* is located on the Z but is absent from the W in this distantly related species which has only a small region of differentiation between the virtually homomorphic Z and W chromosomes [49]. *DMRT1* is the most conserved gene identified in the vertebrate sex-determining pathway. Heterozygous deletions of the region of human chromosome 9 that contains *DMRT1* results in male to female XY sex-reversal in humans, and mice that carry homozygous deletions of *Dmrt1*

are infertile due to severe defects in proliferation of germ cells [50]. The difference in severity between heterozygous deletions of human and mouse *DMRT1* can be explained by the possibility that mice might represent a farther evolved state away from an ancestral dosage dependent pathway to a stable genetic pathway. Thus *DMRT1* has been associated with sex determination for at least 300 MY in mammals as well as reptiles, and the evidence from the platypus suggests it was the sex chromosome system ancestral to all tetrapods.

3. Sex determination in mammals

The primary function of the sex chromosomes is to determine sex. Other male-specific functions are likely to have been acquired after this primary function evolved and recombination was suppressed. We know that the Y-borne *SRY* gene is responsible for testis determination, but we are only just beginning to understand how it works, how it evolved and what form of sex determining mechanism it replaced.

Classically, there are two steps involved in mammalian sex determination; the genetic step from chromosomes to gonad formation, and the hormone-controlled step from gonad to phenotype. The genetic pathway that results in testis differentiation is controlled by the presence of a master switch (the testis determining factor; TDF). In the absence of this switch, the indifferent gonad later forms an ovary. The embryonic testis brings on the second step by producing gonadal hormones. The production of anti-Müllerian hormone (AMH) in males signals the beginning of the hormonal phase of sexual differentiation, then synthesis of testosterone and its derivatives is responsible for differentiation of nearly all male traits. An exception is the independence of scrotum and mammary development in marsupials, which appears to be due to dosage of a gene on the X, rather than to androgens [52,44].

3.1. Discovery of SRY

A search for the human TDF was conducted over many years by deletion analysis. The region of the Y containing TDF was narrowed to a small region on the short arm, from which *SRY* was isolated [51]. This small, intronless gene had no recognizable features except for a domain that coded for an 80 amino acid DNA-binding and bending domain (called the HMG box because of homology with the High Mobility Group proteins). The mouse orthologue was discovered shortly thereafter, and shown to belong to a whole family of *SOX* (*SRY*-like HMG box-containing) genes. The identity of *SRY* as the human and mouse TDF was confirmed by mutation analysis and transgenesis (reviewed in [52]).

As expected, mouse *Sry* is expressed in the somatic cells of the indifferent gonad just before testis differentiation. Expression must reach a required threshold within a specific window of time for male development to be activated [53,54]. Expression is tightly regulated, starting in the genital ridges at approximately 10.5 dpc and reaching its peak across the whole gonad at 11.5 dpc before declining to undetectable levels at about 12.5 dpc [55].

There has been much speculation about possible roles of *SRY* other than testis determination. There are reports of very early *SRY*/*Sry* expression in the blastocyst and the suggestion has been made that this is responsible for the growth advantage observed for XY embryos in cow and mouse [56,57]. There has been intense interest in the idea that this gene might directly control sex differences in behaviour. Expression of *SRY* mRNA in brain has been reported for human [58] and mouse [59]. It was recently demonstrated that the Sry protein is specifically expressed in tyrosine hydroxylase-expressing dopaminergic neurons of the adult male rodent substantia nigra. Reduced expression of *Sry* in this region of the brain resulted in reduced expression of tyrosine hydroxylase leading to motor deficits in male rats [60], suggesting that *Sry* might directly regulate this protein and the motor behaviours it controls, therefore, playing a role in some sex specific differences of the brain.

3.2. SRY action

The SRY protein contains a domain (HMG box) that binds to DNA at a 6-base consensus target sequence, and bends it through a specific angle. Nearly all sex reversing mutations of human *SRY* occur in the HMG box (reviewed in [61]), implying that the activity of the protein lies in this domain. The products of mutant alleles either bind poorly or bend DNA incorrectly [62]. SRY binds to DNA in the minor groove causing it to bend at specific angles that might bring sequences, or proteins bound to them, into position for activation, suggesting that it acts as a transcription factor (reviewed by [63]). However, the low frequency and recessive pattern of inheritance of human *SRY*-negative XX males [64] and the poor conservation of *SRY* suggested that it might repress a factor that represses maleness, perhaps by repressing a related gene in a double inhibition pathway [65].

It was expected that discovery of the TDF would lead immediately to the other steps in the sex determining pathway, but it is still not clear even what the immediate target of *SRY* is. However, several genes upstream and downstream of *SRY* have been identified through analysis of sex-reversed patients. One of these genes with a vital and conserved role in testis determination is a relative of *SRY*. *SOX9* was discovered on human chromosome 17 near the site of a chromosome rearrangement in patients with campomelic dysplasia, which is accompanied by male to female sex reversal [66,67].

SOX9 is conserved in all vertebrates, and is universally upregulated in the testis and downregulated in the ovary. In human and mouse upregulation precedes *AMH* expression, which signals the hormonal stage of sex determination. A conserved SOX binding site is located 150 bp 5′ of *AMH* and the phenotypic effects of its disruption in mouse indicates that *SOX9* might directly act upon *AMH* [68]. This gene is highly dosage-sensitive in humans. *SOX9* haploinsufficiency results in variable XY sex reversal, amongst other phenotypes (reviewed in [69]), and there is a report of XX sex reversal in human caused by duplication of *SOX9*, indicating that an extra dose of this gene is sufficient to cause male development in the absence of *SRY* [70]. Its importance in mouse sex determination was demonstrated by a *Sox*9 knockout in ex vivo organ culture, in which complete XY sex

reversal was observed [69]. Determining the factor/s that regulate *SOX9* is difficult due to its extremely long regulatory region [71,72], and initial excitement in finding a deletion within this region in a mutant strain of mice with XX male sex reversal ("odd sex") has been tempered by the inability to recreate the phenotype by knocking out this region [73,74].

Although *SOX9* seems to be involved in sex determination in all vertebrates, its upregulation occurs after AMH expression in both chicken [75] and alligator [76], suggesting it acts later in the sex determining pathway of birds and crocodilians than it does in mammals. The situation in fish is complicated by genome duplication, and subfunctionalization of the two copies. In undifferentiated male gonads of zebrafish, one copy (*sox9a*) is expressed in testis, and its upregulation precedes *amh* expression, as for mammal *SOX9*. The other copy (sox*9b*) is expressed in ovaries [77]. This suggests that an early action of *SOX9* before AMH expression represents the ancestral vertebrate condition, although confirmation is needed in amphibians.

3.3. Comparative analysis of SRY

SRY has been identified on the Y chromosome in many placental mammals. In marsupials an *SRY* orthologue was shown to map to the Y chromosome [78], confirming a probable ancestral role for it in mammalian sex determination. However, no *SRY* gene has been discovered in monotremes, despite many searches, and the gene from which *SRY* evolved (*SOX3*) is located on an autosome (Mary Wallis in preparation). Whether another sex determining gene resides on one of the five Y chromosomes of the platypus is unknown, as sequences are yet to be isolated from any of these male-specific elements. *ATRY* (a Y-borne homologue of the sex-reversing ATRX gene in marsupials) is not a candidate since it maps to chromosome 6 [44], and although *DMRT1* is present on the sex chromosomes, its dosage favours females rather than males.

Unexpectedly for a gene with a critical role in the vital task of sex determination, *SRY* is poorly conserved between species [78]. Overall, protein identity decreases with genetic distance between species of placental mammals, although there are striking exceptions. There is moderate conservation of HMG box sequences, but little conservation outside the HMG box (Fig. 3). Within primates there is high amino acid identity along the entire length of the SRY polypeptide, as there is also between the SRY proteins of rat and mouse, and between cow and sheep. In horse the region C-terminal to the HMG box appears to have been stripped down and replaced, or changed at an accelerated rate, sharing only 10–15% amino acid identity with all the other species. The low conservation in this region of horse *SRY* is not explained by a simple inversion and, in the absence of *SRY* sequence from other perissodactyl species, it remains a mystery.

Mouse *Sry* has a strikingly different structure that includes a C-terminal glutamine rich domain, which is absent in *SRY* from humans and other mammals (Fig. 3). Injection of truncated transgenes showed that this polyglutamine domain is critical for XX sex reversal [79], and it was suggested that it acts as a transcriptional trans-activator. The function of this domain is either not required in other mammals, or is filled by different proteins. In *Mus domesticus*, a subspecies of *Mus musculus*, about half of this domain is truncated by a premature stop codon that removes its function as a transcriptional trans-activator [80], whereas in rat

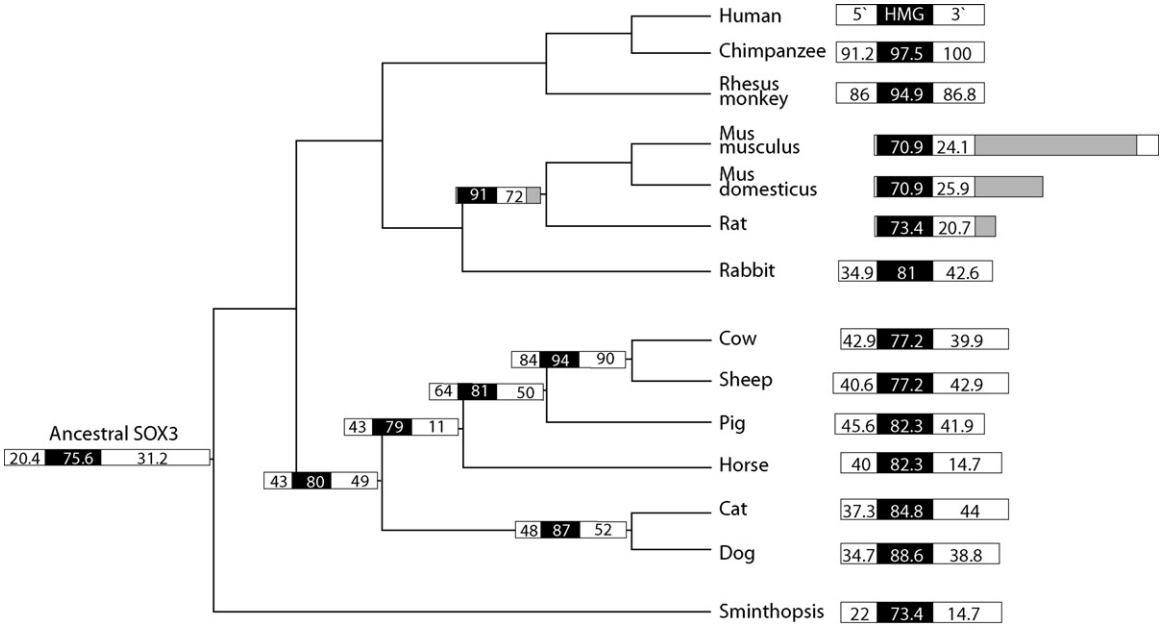

Fig. 3. Alignment of SRY polypeptide from species for which full length sequences are available on NCBI (http://www.ncbi.nlm.nih.gov/). The HMG box is represented in black and flanking regions in white. The glutamine rich domains of rodent *Sry* are indicated in grey. The alignment was constructed using the Clustalx algorithm (http://www.embl.de/~chenna/clustal/darwin/index.html). Indicated within each region of SRY that overlaps with human is the percentage of pairwise amino acid identities for all species compared to human SRY. Percent amino acid identities within clades are indicated by numbers on the branch leading to that clade. The ancestral mammalian SOX3 protein was reconstructed with a consensus sequence of human, mouse, marsupial (*Sminthopsis macroura*), chicken and *Xenopus* SOX3. The highest identities to the overlapping regions of SRY are given within the different sections of SOX3.

the domain is almost completely absent. In rabbit, which is sister to the rodents, there is no evidence of a polyglutamate region. Rabbit *SRY* has the same structure as *SRY* in human and other mammals, and protein identity with human *SRY* is similar to those of other non-primate mammals. Thus the mouse glutamine rich domain was acquired and began to extend after the rabbit and rodent lineages diverged, but before the rat-mouse divergence. When this region was gained, at what rate it expanded and its functional significance in Muridae (and rodents in general) will be clarified when *Sry* is characterized from other rodent species.

A question that has been long debated is whether *SRY* triggers testis development by the same mechanism in all mammals. Initial experiments inserting a human *SRY* transgene into mouse failed to trigger testis determination [81]. However, flanking sequences appear to have cross-functionality, since a transgene introduced into mouse containing various lengths of *SRY* 5′ flanking sequences from pig and human induced expression of a fluorescent reporter gene within the genital ridge of male embryos at the time of sex determination [82]. *SRY* 5′ regulatory signals have been sought by phylogenetic footprinting. Alignment of the *SRY* 5′ flanking sequence across 10 mammalian species reveals overall poor conservation, except for an element near the transcription start point [83]. However, this *SRY* 5′ sequence is surprisingly well conserved within each mammalian group (primates, rodents and bovids) [83].

The cross functionality of *SRY* 5′ flanking sequence is coupled with the surprising cross functionality of the *SRY* gene itself between species. A transgene with the human *SRY* open reading frame inserted into mouse regulatory sequence caused XX sex reversal in mouse [84]. Additionally, a 90 kb transgene containing goat *SRY* caused XX sex reversal in mice for two out of six transgenic lines with expression levels that exceeded the threshold required for sex determination, despite a goat-like expression profile [85]. This suggests that *SRY* action is conserved across species, despite the poor conservation outside the HMG box.

The ability of human and goat *SRY* to reverse sex in a transgenic XX mouse also challenges the hypothesis that the C-terminus glutamine repeats of the mouse *Sry* replace a transcriptional trans-activator function, which is provided by interacting proteins in other mammals [79]. Human and goat *SRY* are active in mouse in the absence of such factors, unless they are still present, perhaps involved in a different pathway.

The *SRY* expression pattern is not entirely conserved between mammals. In mouse, *Sry* expression is confined to a very tight window around the time of testis differentiation, but an inactive circular transcript is expressed in the adult testis [86]. *SRY* in dog, sheep and pig is expressed in the genital ridge at the time critical to sex determination, and expression continues during fetal development in dog and sheep [87,88], although in pig *SRY* expression decreases after testis development is histologically evident [89]. In humans *SRY* is expressed in the genital ridge, then in the testis throughout fetal development, and is also detectable in the adult testis [90]. In marsupials, *SRY* is expressed in many neonatal and adult tissues [91].

These species-specific *SRY* expression profiles might be due to different flanking sequences. A human transgene that contained 5 kb of *SRY* 5′ flanking sequence supported expression within the migrating truncal neural crest cell of both male and female embryos [82]. A pig transgene that contained 4.6 kb of *SRY* 5′ flanking sequence supported expression in the male genital ridge, but not the neural crest, whereas transgenes with 1.6 and 2.6 kb of pig *SRY* 5′ flanking sequence supported expression in the neural crest of both sexes as well as maintaining expression in the male genital ridge. Expression of the reporter gene was lost again from the neural crest of both sexes when 2 kb of mouse *SRY* 5′ flanking sequence (−3 to −1 kb) was placed in front of the 1.6 kb of pig *SRY* 5′ flanking sequence. Therefore, conserved *SRY* 5′ flanking sequences near *SRY* induce expression in the neural crest, whereas this expression is opposed by more distant *SRY* 5′ flanking sequence.

Do different *SRY* expression profiles and different 5′ flanking sequences denote different functional spectra of *SRY* in different species? Or are they relics of an ancient *SOX*3 (the gene from which *SRY* evolved) function in the developing nervous system?

3.4. Evolution of SRY

The ancestral gene from which *SRY* evolved was discovered during attempts to identify marsupial orthologues of *SRY*. It was observed that a human *SRY* probe hybridised to a band on Southern blots that was twice as strong in females as males, implying that it represented an X-borne *SOX* gene [92]. This gene, subsequently identified as *SOX*3, is the *SOX* gene most closely related to *SRY* within the HMG box, so it was proposed that *SRY* evolved from an ancestral *SOX*3 gene on the proto-sex chromosomes [92] (Fig. 3). In this, *SRY* is typical of most of the genes on the Y chromosome, which have paralogues on the X from which they clearly diverged [8].

Mouse *Sox*3 is first expressed in the developing brain and continues throughout the development of brain and central nervous system (CNS) [93,94]. *Sox*3 is then expressed in the developing urogenital ridge, overlapping with *Sry* expression [95], and later in the developed gonad of both sexes [96]. In human, *SOX3* deletion in an XY boy resulted in mild X-linked mental retardation associated with growth hormone deficiency [95]. *Sox*3 mutation in mice has no obvious effect on brain development, but in *Sox*3 knockouts there are mild developmental problems in the gonads of both sexes, and spermatogenesis fails [96,97]. This dual function in brain and gonad is typical of “brains and balls” genes on the X chromosome, mutations in which account for the many mental retardation syndromes accompanied by gonadal abnormalities. Because of its close relationship to *SRY*, and its function in gonads, *SOX*3 was suggested to be the middleman in a double inhibition, repressing *SOX*9 in females, and being repressed by competitive inhibition by *SRY* in males [65]. However, marsupial *SOX*3 is expressed in adult testis and ovary but not urogenital ridge [100], so it could not act in marsupial sex determination, and there is no evidence that *Sox*3 is associated with sex reversal in any mammal so it is unlikely to be directly involved in sex determination.

In many other vertebrates, *SOX*3 is expressed in the urogenital ridge, the developing and adult gonad. *Sox*3 expression has been detected in the immature ovaries of *Xenopus* [101], and it is expressed throughout gonadogenesis in male and female chick-

ens [102]. The conserved expression of *SOX*3 in the urogenital ridge at the time critical to sex determination implies that this was a property of *SOX*3 in ancestral vertebrates.

How did SOX3 evolve into the male-dominant testis determining factor? Human *SOX*3 shows good identity to *SRY* within the HMG box, but little outside it, indicating that the flanking sequences diverged rapidly, or were completely replaced (Fig. 3). Evolution into a dominant sex determining switch was evidently accompanied by loss or mutation of the highly conserved 5′ and 3′ regions. This might have involved mutation to change the target site/s or binding affinity so that it activated key genes (*SOX9*?) in the testis differentiation pathway.

An alternative hypothesis is that *SOX3* lost its status as a transcriptional activator and became a competitive inhibitor of testis-inhibiting genes when its 5′ and 3′ sequences were truncated, just as truncation of *SOX9* reverses its transcriptional activator function [103]. In an ancestral mammal, mutation of one copy of *SOX3* on one member of the proto-sex chromosome pair could have set up a dosage difference that initially controlled testis determination in a dose-dependent manner, as does *DMRT1* in birds. The mutated allele was then truncated to form a male-dominant repressor, whose action is much more robust [65]. The relatively minor phenotypic effects of *Sox3* dysfunction suggest that one allele could be lost or mutated into *Sry* without causing detrimental phenotypic effects.

The expression profile of SOX3 in gonad and brain has largely been retained in *SRY*. Reduced expression of *SRY* in the CNS probably occurred after its specialization as the sex determining factor. Its expression in the brain is likely to be a hangover from the original *SOX3* expression pattern, but may have evidently been retained in parts of the brain for sex-specific functions [60].

We do not know whether the evolution of *SRY* from *SOX3* was the driving force behind differentiation of the mammalian Y chromosome in early mammals, or whether it took over this function from another sex-determining gene. The complete lack of homology between the mammalian XY and the avian ZW sex chromosome systems suggested that they evolved from different autosome pairs in a common ancestor with no sex chromosomes that determined sex by temperature [104]. However, the monotreme sex chromosome complex, containing elements of both systems, suggests a switch from a bird-like ZW system (potentially determined by dosage of *DMRT1*) to the mammalian XY system after a period of meiotic chain formation at male meiosis, as observed in monotremes (reviewed in [45]). But, because the ancestral amniote sex chromosome system is yet to be inferred, the possibility remains that evolution of the bird ZW and the mammal XY is unlinked and, therefore, the platypus meiotic chain evolved after monotreme divergence from other mammals.

4. Conclusion

Decades of studying various mammalian sex chromosome and sex determination systems have shown that mammalian sex determination is not as straightforward as initially proposed [105]. Comparisons between different mammal species, including the distantly related marsupials and monotremes, and even with non-mammal vertebrates, have been critical to our evolving understanding. Features conserved across long evolutionary distance are likely to play important functions that are selected for, and comparing these similarities and differences helps to shed light on sex chromosome origin and evolution.

Sex chromosomes evolve very fast, and mammal sex chromosomes are a relatively recent invention. The X and Y chromosomes of today's mammals originated from an ordinary autosomal pair, which became differentiated as the Y chromosome progressively degraded, losing practically all its active genes except for those with a selectable male-specific function. Most genes on the human Y, even those with known functions in sex and spermatogenesis, evolved from genes on the X that are expressed in brain and gonad.

Surprisingly, the master switch on the Y that determines sex is poorly conserved on the mammal Y chromosome, although downstream genes are relatively well conserved. However, the SRY protein and its regulatory sequences function across species, indicating that although the protein has changed significantly, its action has not. Variability in the sex determining switch is even more pronounced across different vertebrate classes, with unrelated genes performing this function in some groups, and environmental cues in others.

Further analysis of sex chromosomes and sex determination in different mammals and vertebrates (especially previously uncharacterised taxa that fill interesting evolutionary gaps) will continue to provide exciting and novel insights into their evolution. Of particular value would be complete sequence characterization of the Y chromosomes from a range of mammals, so that the methods of comparative sequence analysis that have proved so powerful for analysing mammalian X and autosomes can be extended to, perhaps the most fascinating element of the mammal genome, the Y chromosome.

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