Evolution of the Mammalian Y Chromosome and Sex-Determining Genes

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ABSTRACT In mammals, male sex determination, as well as spermatogenesis, is controlled by genes on the Y chromosome. Evolutionary comparisons may be used to detect and test candidate genes for these functions, under the hypothesis that the rapid evolution of the mammalian Y chromosome causes it to contain few genes other than those with a critical function in male reproduction. Comparisons of the gene content of sex chromosomes from the three major groups of extant mammals (placentals, marsupials, and monotremes) show that part of the X chromosome, and a corresponding region of the Y, is shared by all mammals and must be very ancient, but part was added relatively recently. Evolution of the mammalian Y took place in several cycles of addition and attrition, as autosomal regions were added to the pseudoautosomal region of one sex chromosome, recombined onto the other, and degraded on the Y. This explains why most genes and pseudogenes on the Y chromosome have relatives on the X. The gene *SRY* itself is apparently no exception, being closely related to the highly conserved X-linked gene *SOX*3. Comparisons of *SRY*/*SOX* base sequence and gene location in the three groups of mammals suggest that *SRY* evolved from *SOX*3 relatively recently by mutation and loss of all sequences outside the HMG box. It is suggested here that, rather than acting as a transcriptional activator, the *SRY* gene acts to inhibit its paralogue *SOX3*, which in turn inhibits an ancient autosomal sex-determining gene *SOX9*. *J. Exp. Zool. 281:472–481, 1998.* © 1998 Wiley-Liss, Inc.

Sex determination in mammals is accomplished by a chromosomal mechanism, females having two X chromosomes, and males a single X and a Y. The presence of a Y chromosome determines a male phenotype, no matter how many copies of the X are present, and this has been ascribed to the presence of a dominant "testis-determining factor," TDF.

Genes on the Y chromosome share a number of characteristics that set them apart from genes on the X and the autosomes. Their origins have been deduced by comparisons between distantly related mammals, which show that the mammalian Y evolved from the X by cycles of attrition, offset by addition of autosomal regions. Genes on the Y, therefore, derived either from the original X or, more recently, from one or other of the added regions. The only genes that survived on the Y chromosome for any length of time evolved male-specific functions in sex determination and spermatogenesis. Their structure and function have been imposed by the evolutionary forces that shaped the Y.

Of special interest is the testis-determining factor on the Y, which was identified as the *SRY* gene 7 years ago. This gene has been assumed to act as a transcriptional activator, turning on downstream testis-differentiating genes. Comparative studies suggest, however, that *SRY* may act indirectly, by repressing another gene in the pathway. Here I suggest that *SRY* interacts with related genes to control the activity of an autosomal testis-determining gene.

GENE CONTENT OF MAMMALIAN SEX CHROMOSOMES

The human X chromosome amounts to about 5% of the haploid genome and accounts for about this proportion of genes. Many of these 3,000-odd genes were identified by the phenotypes of boys with mutant alleles, and more than 300 have been cloned. They represent a rather average mix of classic housekeeping enzymes and products with specialized functions like visual pigments or blood-clotting factors. The X is highly conserved between

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different species of eutherian ("placental") mammal, perhaps because of the chromosome-wide inactivation mechanism that ensures dosage compensation between males and females.

The human Y chromosome is much smaller and heterochromatic. It recombines with the X only over a tiny "pseudoautosomal region" (PAR) at the tips of the short arms. It contains only three genes recognizable by their phenotypes: the testis-determining factor, TDF; a minor male-specific antigen, HYA; and the azoospermia factor, AZF, a region thought to contain a spermatogenesis gene because deletion confers sterility in azoospermic men. Several genes and pseudogenes have been isolated from cloned regions of the Y or by homologous cloning using probes that map elsewhere on the genome. A remarkable feature of these genes is that almost all of them detect an obvious homologue on the X chromosome (reviewed by Graves, '95).

Unlike the X, the Y is poorly conserved between species. Some genes, such as *RPS4Y* and *DAZ,* are present on the human Y but absent from the mouse Y. *Ube1y* has the reverse pattern, being present on the Y of all eutherian mammals except primates. Several Y-borne genes are active in one species and inactive in others; for instance *AMEL* is active in human but is a pseudogene in mouse, whereas *Sts* is active in mouse but represented by a pseudogene on the human Y. The gene content of the PAR is not conserved, but represents different subsets of markers present on the short arm of the human X (human Xp) in primates, carnivores, artiodactyls, and mouse. The content and activity of genes on the Y chromosome has therefore changed rapidly during recent eutherian evolution.

Evolution of the mammalian sex chromosomes has been approached by looking for variation of sex chromosomes among the three major extant groups of mammals. Marsupials and monotremes represent the mammals most distantly related to humans and mice, having diverged from eutherians about 130 and 170 million years ago, respectively, early in the 200-million-year history of class Mammalia. The basic marsupial X is smaller than the eutherian X, and the Y can be as small as 12 Mb. The X and Y seem not to undergo homologous pairing at meiosis and presumably do not share a pseudoautosomal region. The monotreme sex chromosomes are large, and the X and Y pair at meiosis over the entire short arm of the X and long arm of the Y.

Comparative mapping, using somatic cell genetics and in situ hybridization, has shown that the X chromosome of marsupials includes all the genes on the long arm and the pericentric region of the human X (reviewed in Graves, '95). Because the same suite of genes lies on the monotreme X, it must represent a highly conserved original mammalian X (XCR). The marsupial and monotreme X, however, lacks the genes on the rest of the short arm of the human X. Since marsupials and monotremes diverged independently from eutherians, the most parsimonious explanation is that this region was recently added to the X in the eutherian lineage (Fig. 1). The observation that genes within this added region (XAR) map to three similar clusters in marsupials and monotremes suggests that there have been at least three additions to the X.

The Y chromosome of marsupials shares at least four genes with the Y of humans and/or mice. *SRY, RBM1, SMCY* and *UBE1Y* have been cloned and mapped to the marsupial Y (Sinclair et al., '90; Mitchell et al., '92; Delbridge et al., '97; Duffy et al., in prep.). A number of genes within the re-

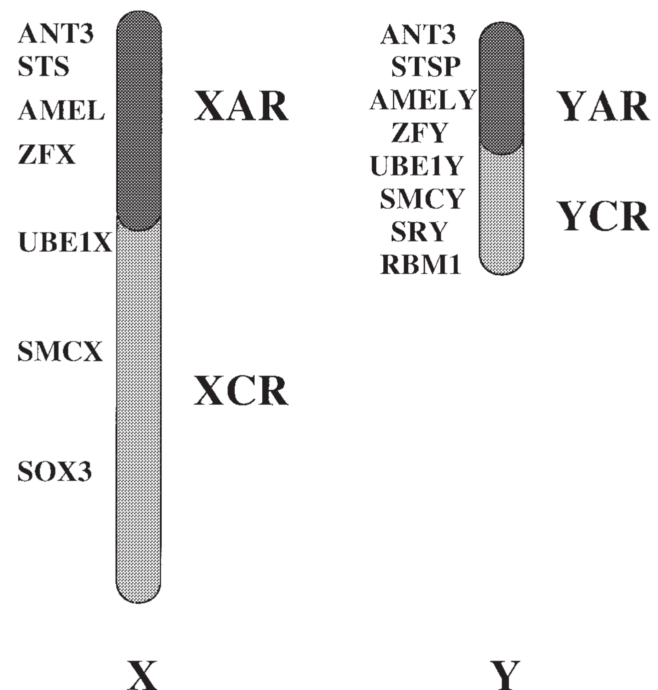

Fig. 1. The evolutionary origins of regions of eutherian sex chromosomes. The differential region of the X chromosome consists of a conserved region XCR (shaded), shared by the X of all mammals, and an added region XAR (white), which is on the X only in eutherians. The differential region of the Y also consists of a conserved region YCR and a recently added region YAR. XY-shared genes which define these regions are shown.

cently added region have homologues on the human X and Y, including human and mouse pseudoautosomal genes. These genes detect only autosomal sequences in marsupials (Toder and Graves, '98), indicating that the region was recently added, not only to the eutherian X, but also to the eutherian Y, defining a YCR and a YAR (Fig. 1). A hypothesis has been presented in which autosomal regions have been progressively added to the X above the PAR and recombined onto the Y by exchange within the PAR, extending the PAR in stages; the initial addition could equally well have been to the Y, with recombination onto the X (Graves, '95). One way or the other, the X and the Y grew incrementally during eutherian, but not marsupial, evolution.

THE ORIGIN AND FATE OF THE MAMMALIAN Y CHROMOSOME

The observations that most or all of mammalian Y-borne genes have X-linked homologues and that the eutherian X and Y share a homologous pseudoautosomal region confirm that the X and Y evolved from a pair of autosomes in an ancestral mammal. This hypothesis was proposed long ago by analogy to snake sex chromosomes, which are thought to represent a series of states intermediate between undifferentiated and strongly differentiated Z and W chromosomes (Ohno, '67). It receives support from comparative studies of mammal and bird sex chromosomes. Like snakes, birds determine sex by a ZZ male/ZW female sex chromosome system (female heterogamety). Limited mapping of genes homologous between birds and mammals reveals none shared between the conserved mammal X and the bird Z (Table 1), indicating that the two sex chromosome systems evolved independently from different autosomal pairs. Other vertebrate classes show a wide variety of genetic and environmental sex determination, so that it is not possible to deduce the sex-determining system of the common reptilian ancestor.

Differentiation of the X and Y chromosomes is thought to have been initiated in an ancestral mammal when an allele at a single locus on the proto-Y took over a male-determining function from an ancestral genetic or environmental sex-determining system. The evolution of a second sex-determining or differentiating locus on the proto-Y then set up conditions where the co-inheritance of these alleles would present an advantage to males, and this selected for suppression of recombination between the two loci. Accumulation of other genes with a male-specific function within this protected region would be an advantage, and would lead to the enlargement of this non-recombining region by chromosome rearrangement and heterochromatinization. Once a region becomes genetically isolated from its homologue, mutations, insertions, and deletions accumulate either by genetic drift, or by selection of a new allele in the non-recombining region (Charlesworth, '91). Comparison of sequences of X-Y shared genes across species shows that the Y copy changes far more rapidly than the X (Tucker and Lundrigan, '93; Whitfield et al., '93).

The autosomal regions added to the mammalian Y have also been degraded, though more recently. Several genes like ZFY in the recently added regions of the X still have active homologues on the Y, whereas others, like *STS* and *KAL,* detect pseudogenes whose structure and sequence suggest very recent divergence from the X-linked allele. *PDB* straddles the pseudoautosomal boundary, being identical to the X-borne allele at one end and truncated at the other. Thus, attrition of Y-borne sequences removes most of the genes

TABLE 1. Genetic non-homology of the sex chromosomes in humans and birds[1]

Locus	Name	Location in humans	Location in birds
PGK1	phosphoglycerate kinase	Xq	4
G6PD	glucose-6-phosphate dehydrogenase	Xq	Autosomal in pigeon
HPRT	hypoxanthine phosphoribosyltransferase	Xq	Micro?
DMD	dystrophin	XAR	10
OTC	ornithine transcarbamylase	XAR	Z
COLA1A1	collagen type 1 alpha 1	17q	Z
COL1A1B	collagen type 1 beta 1	17q	Z
GHR	growth hormone receptor	5p	Z
IREB1	iron response element binding protein 1	9	Z
ACO1	aconitase 1	9	Z
CK	creatin kinase	19	Z

[1]Bird chromosome locations are in the chicken unless otherwise specified.

within added autosomal regions, as well as within the original Y.

Progressive inactivation and loss of alleles from the Y chromosome would impose gene dosage differences between males and females were it not for the spread of inactivation along the X, which more-or-less keeps pace with Y attrition. Thus, most genes in the recently added region of the X are subject to inactivation. Exceptions are the several human X-Y shared genes, which escape from inactivation, presumably because there is no dosage difference between males and females. However, there are several genes whose activity states show that Y attrition and X inactivation may be out of step. Human *UBE1X* escapes inactivation, although *UBE1Y* has been lost from the *Y*. Human *STS* is only partially inactive, although its Y homologue is now an inactive pseudogene, and mouse *Sts* is partially inactivated, although it is still pseudoautosomal. The loss of *UBE1Y* from the human Y and the inactivation of *STSP* are likely to have been very recent events, implying that there has simply been insufficient time for X inactivation to catch up with Y attrition. Evidently, dosage differences of most genes may be tolerated for a few million years.

POSSIBLE FUNCTIONS OF GENES ON THE MAMMALIAN Y CHROMOSOME

The inexorable degradation of the Y chromosome makes it a risky place for a gene to be, and most genes on the Y have been mutated, inactivated, and deleted within the last hundred million years or so. The few that have survived on the Y are therefore a select group.

What selective forces would act to retain active genes on the Y, against progressive degradation? Conceivably, a gene might be dosage sensitive, so that even a transient imbalance caused by loss from the Y is lethal. There are well-known examples in which haploinsufficiency for an autosomal gene (e.g., the recently cloned *SOX*9 gene) is deleterious or lethal. If this were the case, however, we would expect that X and Y-borne genes should be complementary and the original sequence and function of the Y allele should have been retained. The opposite seems to be the case.

The alternative is that Y-specific genes are retained for long periods because they each acquire a unique selectable function. Because females lack a Y chromosome, the only important functions such a gene could serve are those that are critical for male determination or differentiation. This idea receives support from characterization of the only four genes shared by the marsupial and eutherian Y, *SRY, UBE1Y, RBM1,* and *SMCY*, which must therefore have been on the Y for at least 130 million years. Three of these genes have gonad-specific expression patterns in mouse and are thought to function in sex determination (*SRY)* or spermatogenesis (*UBE1Y, RBM1* and *SMCY*). The recently added *ZFY* and *DAZ* are expressed in the testis and thought to function in spermatogenesis. These genes are evidently selectable. Other genes on the eutherian but not the marsupial Y (the recently added *AMEL*, *KAL,* and *STS*) do not perform male-specific functions and seem to be disposable. Even the acquisition of a male-specific function may not save genes on the Y from eventual degradation and loss. *Ube1y* has been recently lost in the primates, although it is thought to have a function in spermatogenesis in the mouse.

Genes on the mammalian Y chromosome seem to represent a small, nonidentical but overlapping subset of genes on the X. Some apparently expendable genes seem to be dead or dying, whereas others appear to serve a male-specific function that ensures their survival over long periods of evolutionary time. The cloning and characterization of the *SRY* gene and its relatives suggest that the *SRY* gene itself acquired its testis-determining function in this haphazard manner.

THE SEX-DETERMINING GENE ON THE Y CHROMOSOME

The sex-determining function was first ascribed to the human Y chromosome as a result of the phenotypes of individuals with aberrant sex chromosomes. It was later mapped to the distal region of the short arm of the human Y by painstaking deletion analysis, using DNA from "sex-reversed" humans with fragments of a Y chromosome. *ZFY* was initially considered a candidate gene (Page et al., '87) but was eliminated, partly because of its lack of conservation on the Y in marsupials (Sinclair et al., '88). Ultimately, more refined mapping defined a new minimum sex-determining region of the human Y, which lacked *ZFY*, just proximal to the pseudoautosomal region, and the *SRY* gene was cloned from this new region (Sinclair et al., '90). The finding of *SRY* mutations in several human XY females supported the proposition that this gene represented TDF (Hawkins, '93). The human *SRY* gene detected male-specific homologues in many placental mammals, and a related gene was cloned from marsupials and shown to map to the Y (Foster et al., '92). The mouse ho-

mologue *Sry* was shown to be expressed in the genital ridge just before testis differentiation, and its identity as the testis-determining factor was irrefutably demonstrated by the male development of XX mice transgenic for *Sry* (Koopman et al., '90, '91).

SRY is a small, intronless gene coding for a protein with homology to an 80-amino acid "HMG box" shared by many proteins, including the high-mobility group. Biochemical studies of *SRY* protein showed that the HMG box 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., '92; Harley et al., '92). Because of its male-dominant effect on sex determination, it was expected that *SRY* would prove to code for a transcription factor that activated downstream genes in a conserved testis differentiation pathway.

Cloning and characterization of marsupial *SRY* produced major surprises. Unexpectedly for a putative transcription factor critical for reproduction, *SRY* sequences were remarkably variable. Within the "conserved" HMG box, mouse and human *SRY* gene products share homology of only 71%, and marsupial and human only 63%. Outside the box, mouse and human *SRY* genes have completely nonhomologous 5´ and 3´ coding regions. Equally puzzling were the differences in *SRY* expression patterns in different species. *SRY* is expressed specifically in the genital ridge in mouse (Koopman et al., '91), has wide expression in embryonic and adult human tissues, and is virtually ubiquitous in marsupials (Clepet et al., '93; Harry et al., '95).

Another surprising finding from investigations on the marsupial *SRY* was that this gene, like others on the Y chromosome, has a related sequence on the X. Initially it was reported that the human *SRY* probe detected several related autosomal "*SOX*" (for *SRY*-like HMG box-containing) sequences (Gubbay et al., '90; Sinclair et al., '90). Cloning and mapping of some of the male-female shared bands detected in marsupial DNA by *SRY* revealed that *SOX3* maps to the X chromosome in marsupials, and this was later found to be the case for human and mouse *Sox3*. Unlike *SRY*, *SOX3* shows almost 100% conservation among human, mouse, and marsupial, and curiously, the *SRY* genes of these three groups are all more closely related to *SOX3* than they are to each other (Foster and Graves, '94). This implies that *SOX3* is the ancestor of *SRY,* and suggests that the evolution of *SRY* followed exactly the same pathway as for other genes on the Y that evolved from X linked homologues and acquired a male-specific function.

The nature of this function is still not at all certain. The dominant testis-determining function of the mammalian Y chromosome early led to the expectation that TDF acts to activate downstream genes in a linear testis differentiation pathway (e.g., Werner et al., '96). With the isolation and characterization of the *SRY* gene, it was expected that identification of these downstream genes and the nature of these interactions would be straightforward. However, although it is clear from mutation analysis and transgenesis that *SRY* is necessary and sufficient for testis determination, it is far from clear how it acts to switch the gonad differentiation pathway into the male direction.

FUNCTION OF THE TESTIS-DETERMINING FACTOR

SRY has been suggested to act as a transcriptional activator at the core of a sex-determining pathway. Other SOX genes are transcriptional activators (e.g., *Sox4;* van de Wetering, '93), and *SRY* does show transcriptional activity in vitro (Cohen et al., '94). There is a potential transactivating domain in the mouse *SRY* gene (Dubin and Ostrer, '94) and a PDZ domain in the C-terminal of the human *SRY* that might mediate interactions with transcription factors or other related genes (Poulat et al., '97).

The HMG box of *SRY* and other HMG proteins binds to DNA and bends it through a specific angle (Ferrari et al., '92). Binding and bending of human *SRY* protein appears to be critical to its function, because several sex-reversing mutants of *SRY* show altered binding or bending (Rimini et al., '95). Human *SRY* binds to DNA in the minor groove at a 6-base consensus sequence AATAAG and bends it through 65°, and mouse *SRY* binds in the major groove and bends DNA through 80°. Bending has been proposed to act to bring flanking DNA sequences or the proteins that bind them into juxtaposition to promote transcription of the target gene. This target consensus sequence has been found in some interesting genes, including *MIS* (Mullerian Inhibiting Substance, one of the first products of the testis, which causes regression of the female ducts), and in vitro experiments support the idea that *SRY* protein may directly activate these downstream genes (Haqq et al., '93). Alternatively, binding with *SRY* was found to enhance transcription of the *fra-1* promotor, suggesting that it might

function by activating more general transcription factors (Cohen et al., '94).

Several authors have put forward sex-determination pathways in which *SRY* directly activates downstream genes involved in testis differentiation and function, such as MIS, and in which *SRY*, in turn, is under the control of upstream genes, such as the steroidogenic factor SF1 or DAX1, a candidate for the dosage-sensitive sex reversing gene on the X (e.g., Lovell-Badge and Hacker, '95; Luo et al., '95; Jiminez et al., '96; Swain et al., '96; Werner et al., '96). Alternatively, it has been suggested that *SRY* is only peripherally involved in the sex-determination pathway (Graves, '95) or acts as an inhibitor of an inhibitor (McElreavey et al., '93).

Indeed, the function of *SRY* as a transcriptional activator is far from established, and several observations are more easily reconciled with the idea that it acts as an inhibitor. The lack of conserved sequence outside the box, and the location of nearly all the sex-reversing mutations within the box, implies that all the activity lies within the box. Stripped of its transactivation domain, the related *SOX9* gene becomes an inhibitor (Sudbeck et al., '96). The poor sequence conservation even within the box, and the interspecies difference in the *SRY* binding and bending of DNA, are unusual for transcriptional activators, which are generally highly conserved. *SRY* could therefore function as an inhibitor, as suggested by McElreavey et al., ('93). A likely target of *SRY* inhibition is a related SOX gene that shares the same binding site (Graves, '98).

The *SOX* gene family includes more than 20 representatives, all highly conserved between species within and outside of the box. They have important development functions in both sexes. For instance, *SOX1, 2,* and *3* are expressed in central nervous system, and *SOX9* is critical for chondrogenesis (Collignon et al., '96; Bell et al., '97; Lefebre et al., '97). It is now clear that *SOX9* is also involved in vertebrate sex determination. This gene was cloned after a search for the gene involved in campomelic dysplasia and associated sex reversal (Foster et al., '94; Wagner et al., '94). It is highly expressed in the developing gonad in XY male (but not XX female) mouse embryos. Significantly, it is also differentially expressed in developing gonads in ZZ male (but not ZW female) chick embryos (Kent et al., '96; Morais da Silva et al., '96), and is also differentially expressed in the genital ridge of male and female alligators, which have no sex chromosomes (Western et al., unpublished). This conservation of a male-specific expression pattern in vertebrates with entirely different sex chromosome systems strongly implies that *SOX9* is involved in normal sex determination in all vertebrates.

Does *SRY* regulate *SOX9*, or does *SOX9* regulate *SRY*? The observation that upregulation of *SOX9* in XY embryos coincides with *SRY* expression was interpreted as evidence that *SRY* activates *SOX9*, although activation of *SRY* by *SOX9* has also been proposed (e.g., Werner et al., '96). However, if *SRY* is an inhibitor, any kind of direct interaction with *SOX9* is logically difficult to sustain because *SOX9* and *SRY* both act in a positive way in testis determination.

This problem could be overcome if a third gene were involved in the sex-determining circuitry. *DAX-1* on human Xp has been suggested because it is sex-reversing when duplicated and might have had a dosage-regulated role in sex determination in an ancestral mammal. However, this gene is autosomal in marsupials (Pask et al., '96), so was not originally on the X. I have proposed that *SRY* and *SOX3* interact to regulate *SOX9* action as a testis-determining trigger (Graves, '98). *SOX3* is a highly conserved and closely related sequence on the X from which *SRY* is thought to have evolved as the sex chromosomes diverged. Specifically, I suggest that *SOX3* inhibits *SOX9* function in females, but in males, *SRY* inhibits *SOX3* and permits *SOX9* to enact its testis-determining role (Fig 2). This could occur by the interaction of *SRY* and *SOX3* in the transcriptional regulation of *SOX9*. Although the hypothesis does not prescribe the level at which inhibition operates, the expression patterns of the three genes are quite compatible with this hypothesis, which could account for the curiously inconsistent patterns of *SRY* expression in different species. *Sox9* is expressed in gonads of both XX and XY mouse embryos at day 10.5. It is upregulated in XY embryos at the onset of *Sry* expression and remains level or decreases in XX embryos. This pattern is compatible with inhibition of *Sox9* by *Sox3* in XX embryo and relief of this inhibition by competition with *Sry*, which is expressed at the same time (Koopman et al., '91) in XY embryos. *Sox3* expression occurs at about the same time in the genital ridge of both sexes (Collignon et al., '96); this expression is compatible with such an inhibitory role.

The hypothesis that the testis-determining action of *SOX9* is regulated by *SOX3* and *SRY* makes a number of testable predictions about the sexual phenotype of humans or other mammals with mutations or changes in the dosage of any

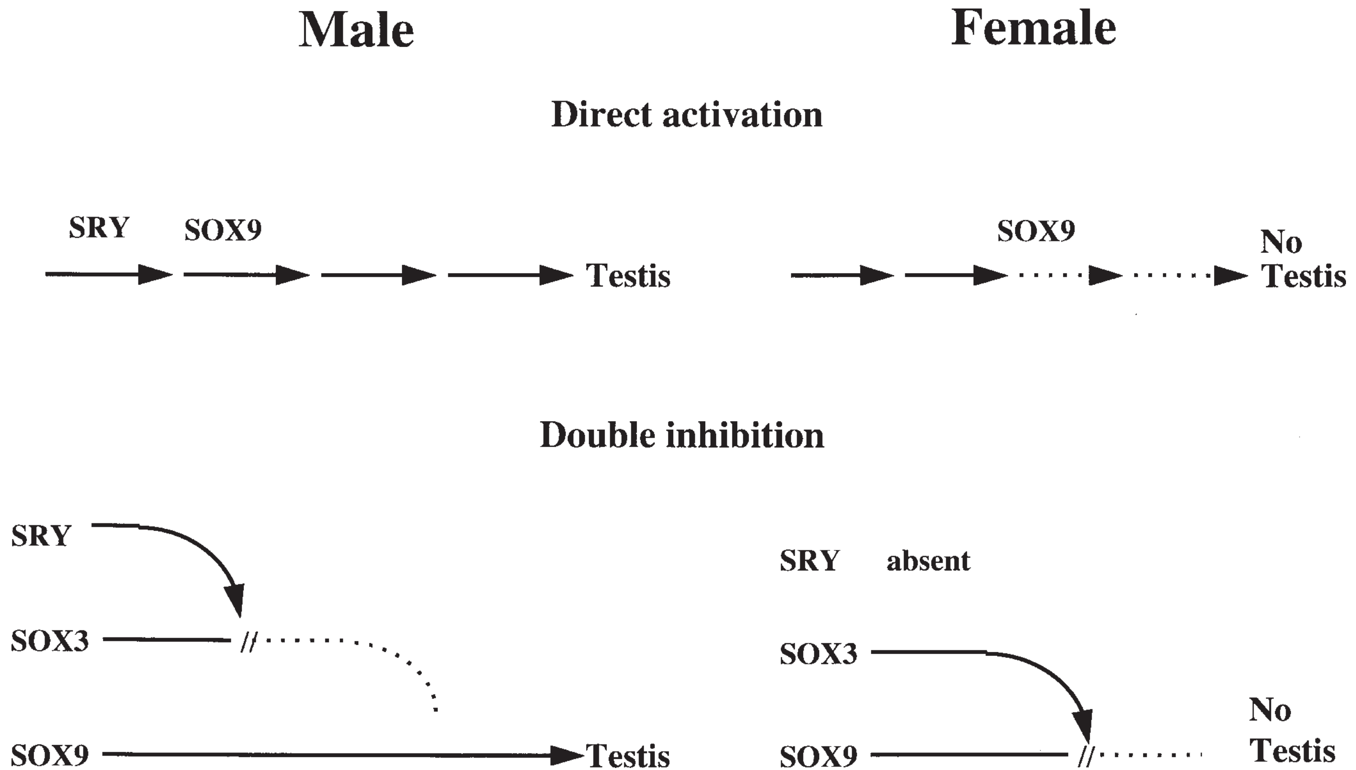

Fig. 2. Hypothesis that sex determination is controlled by the interaction of *SRY* and *SOX* genes. The product of autosomal gene *SOX9* is required to determine testis. In females, *SOX9* is inhibited by product of related X-borne gene *SOX3*, and no testis forms. In males, *SOX3* is inhibited by the product of its Y-borne paralogue *SRY*, so that *SOX9* is able to function and determine testis.

of these three genes (Graves, '98). Of the twelve possible genotypes, seven have been observed, and all are consistent with the predictions. Sex reversal in XY females (the majority of whom have a normal *SRY* allele) could result from a deficiency of *SOX9*, or a duplication or overproduction of *SOX3*. In particular, double inhibition easily accounts for XX(*SRY*-) males, which are otherwise difficult to explain without invoking constitutive gain-of-function mutations (McElreavey et al., '93). These XX males could arise by deletion or mutation of *SOX3*, or by duplication or overproduction of *SOX9*. It should be possible to search for such mutations in the small *SOX3* and *SOX9* genes. The hypothesis could also be tested by constructing *Sox3* and *Sox9* knockout and transgenic mice.

The hypothesis could also account for sex-reversed phenotypes in some mammal species with aberrant sex chromosomes. For instance, the curious X* chromosome, which is sex reversing in X*Y female wood lemmings (Fredga, '88) may contain a mutated or duplicated *SOX3*. Similarly, altered balance of *SRY*, *SOX3,* and *SOX9* may account for conditional *SRY* mutations (Hawkins, '93), as well as for the development of testicular tissue in foetal ovaries in culture or grafted to the kidney capsule of adult XY or XX mice (Taketo-Hosotani et al., '85), where the central nervous system-specific *SOX3* is not expressed.

Obviously, many other autosomal genes are involved in mediating the action of *SRY* and *SOX9* (Eicher et al., '96). However, the close relationship between SRY and SOX9 suggests that there is some interaction between *SRY* and *SOX* genes. This interaction may be more easily understood in terms of its evolutionary history than its function.

EVOLUTION OF *SRY* AND THE SEX-DETERMINING PATHWAY

SRY may therefore act indirectly to control testis determination, the male-dominant action of the testis-determining factor notwithstanding. It is instructive to consider how *SRY* may have evolved to take on this role.

The association of *SOX9* with sex determination in birds and reptiles as well as mammals implies a role in a common ancestor at least 350 million years ago. In birds and reptiles in which

sex chromosome systems are entirely non-homologous or absent and there is no sex-specific *SRY*, *SOX9* may be regulated by an entirely different control pathway. The basic steps of the gonad differentiation, including that regulated by *SOX9*, are likely to be identical, however. Initially, *SOX9* may have been part of an ancestral vertebrate sex-determination pathway. Male-dominant mammalian sex determination might have evolved through an intermediate stage in which *SOX9* was regulated by dosage of *SOX3* on a differentiating sex chromosome pair, rather than by inhibition by *SRY*. The degeneration of *SOX3* on the proto-Y into a truncated *SRY,* which blocked the inhibiting action of *SOX3,* subsequently reinforced and ultimately replaced this dosage-regulated system for determining sex in mammals (Graves, '98).

Far from being remarkable, the changes in *SRY* structure and function are typical of genes on the Y chromosome. They can be understood in terms of the addition-attrition hypothesis of sex chromosome evolution, which proposes that, when the Y became isolated from recombination, genes on the proto-Y chromosome and additions to it were either mutated and lost, or remained, either because they have been added recently and have not yet had time to be degraded and lost, or because they have each taken on a unique male-specific function that ensure its survival. Like the other genes *SMCY, RBM1,* and *Ube1y* that have been on the Y chromosome for at least 130 million years, *SRY* was retained because it evolved a male-specific function.

The structure and expression of *SOX3* has changed during its evolution into *SRY*. Like other genes on the Y that have acquired male-specific functions, *SRY* shows high rates of mutation, amplification, and loss. *SOX3* specifies almost identical products in human, mouse, and marsupials, whereas *SRY* has changed rapidly in rodent, primate, and marsupial evolution and is almost unrecognizable in more distantly related mammals (Foster et al., '92; Tucker and Lundrigan, '93; Whitfield et al., '93; Pamilo and O'Neill, '97). Mutation, internal deletions, and transpositions may explain the complete non-homology of sequences outside the HMG box. *SRY* also shows the full range of changes noted in other Y-borne genes, including amplification and loss. In several Old World mouse species, *SRY* has been amplified. Most remarkably, just as primates lack *Ube1y*, mole vole species that lack a Y chromosome have been shown to lack *SRY* (Just et al., '95). Presumably, another sex-determining gene on an autosome has short-circuited the sex-determining process, and the whole sequence of attrition is about to start over again.

The evolution of *SRY* from *SOX3* was also accompanied by changes in its expression pattern. Mouse *Sox3* is expressed in the central nervous system as well as the genital ridge, whereas *Sry* product is expressed only in the latter. This parallels the ubiquitous expression pattern of mouse *Zfx* and *Ube1x,* which evidently have important general roles in both sexes, compared with the gonad-specific pattern of expression of the Y-borne partners *Zfy* and *Ubey* (reviewed in Graves, '95). Human *SOX3*, as well as *SRY*, has a wider pattern of expression.

Thus, the evolution of *SRY* has followed the same haphazard course as has the evolution of other genes on the Y. Like other Y-borne genes, it 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 that protected it from inactivation and deletion. *SRY* acts, directly or indirectly, with a complex pathway that makes evolutionary, if not functional sense.

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