Interactions between SRY and SOX genes in mammalian sex determination

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Summary
The SRY gene on the mammalian Y chromosome undoubtedly acts to determine testis, but it is still quite unclear how. It was originally supposed that SRY acts directly to activate other genes in the testis-determining pathway. This paper presents an alternative hypothesis that SRY functions indirectly, by interacting with related genes SOX3 (from which SRY evolved) and SOX9 (which appears to be intimately involved in vertebrate gonad differentiation). Specifically, I propose that in females SOX3 inhibits SOX9 function, but in males, SRY inhibits SOX3 and permits SOX9 to enact its testis-determining role. This hypothesis makes testable predictions of the phenotypes of XX and XY individuals with deficiencies or overproduction of any of the three genes, and is able to account for the difficult cases of XX(SRY−) males and transdifferentiation in the absence of SRY. The hypothesis also suggests a way that the dominant SRY sex-determining system of present-day mammals may have evolved from an ancient system relying on SOX3 dosage. BioEssays 20:264–269, 1998.

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between human, mouse, and marsupial SRY, suggesting that DNA binding is the sole conserved role of the SRY gene product. This conclusion is reinforced by the finding that almost all the known amino acid substitutions found in mutant SRY genes from XY females lie within the HMG box. Even within the HMG box, sequence homology between species is much lower than for most transcription factors, which are generally very conserved, and the HMG box bends DNA through quite different angles in different species. A lack of conservation of sequence and function may be more compatible with a role for SRY as a competitive inhibitor that recognizes and blocks the same relatively simple DNA binding site. Indeed, loss of the C-terminal transactivating domain of SOX9 protein produces just such inhibition.

An inhibitory role for SRY product was previously suggested to account for the puzzling cases of XX males who lack SRY. These rare cases are difficult to explain without invoking constitutive gain-of-function mutations in other (unknown) genes in the sex-determining pathway. Equally awkward for theories of a positive action for SRY are the observations of induction of testicular tissue in foetal ovaries that are grown in culture or grafted to the kidney capsule of adult XY or XX mice.

It is clear that other genes are active in a sex-determining pathway in mammals, but it has been difficult to identify them. Some leads are provided by sex-reversal syndromes in humans. Campomelic dysplasia, characterized by a severe bowing of the leg bones in children with a deletion or rearrangement of part of chromosome 17, is often accompanied by male-to-female sex reversal. SOX9, a gene related to SRY, which was previously cloned in mice and shown to be expressed in cartilage, was found to be deleted or mutated in these cases.

The recent finding that SOX9 is expressed in the developing gonad in XY male (but not XX female) mouse embryos suggests that SOX9 is involved in testis determination in males, as well as cartilage determination in both sexes. The observation that SOX9 is also differentially expressed in the male, but not the female, gonad in chick embryos strongly implies a conserved role in vertebrate sex determination that is independent of the sex chromosome system. The further observation that the upregulation of SOX9 in XY mouse embryos coincides with SRY expression has been interpreted as evidence that SRY activates SOX9, but activation of SRY by SOX9 has also been proposed.

However, if SRY is an inhibitor, any direct interaction with SOX9 is logically difficult to sustain, as both act to promote testis determination. This problem could be overcome if a third gene is involved in the sex-determining circuitry.

THE HYPOTHESIS

This paper proposes that SRY and SOX3 interact to regulate SOX9 action as a testis-determining trigger. Specifically, I suggest that in females SOX3 inhibits SOX9 function, but in males, SRY inhibits SOX3 and permits SOX9 to enact its testis-determining role (Fig. 1).

Since haploinsufficiency of SOX9 promotes ovarian, rather than testicular, differentiation, inhibition of SOX9 in genital ridge must suppress testis formation and lead to a female phenotype. Obviously, SRY can have no such direct inhibitory effect, since SRY presence promotes, rather than inhibits, testis. The gene that provides a link between SRY and SOX9 is suggested to be SOX3, a highly conserved and closely related sequence on the X, from which SRY is thought to have evolved as the sex chromosomes diverged. SOX3 is expressed in the central nervous system (CNS) in mouse embryos, and its deficiency does not cause male-to-female sex reversal. I previously proposed that SRY could act by competitively inhibiting the closely related SOX3 gene; now I suggest that SOX3, in turn, inhibits the action of SOX9. The opposite action of related genes would be by no means unique, since other families of transcriptional regulators (e.g., Sp1) contain activators and inhibitors.

This hypothesis does not prescribe the level at which inhibition operates, though the expression patterns of the three genes are quite compatible with a hypothesis that SRY and SOX3 interact in the transcriptional regulation of SOX9. Indeed, indirect action may account for the curiously inconsistent patterns of SRY transcription between species. SRY is expressed appropriately in the genital ridge of day 10.5 mouse embryos, but it has surprisingly wide expression in humans and is virtually ubiquitous in marsupials. If the
action of SRY were indirect, as proposed, the expression pattern of its target may be more relevant than its own.

SOX9 is expressed in gonads of both XX and XY mice at day 10.5. It is upregulated in XY embryos at the onset of SRY expression, and remains level or decreases in XX embryos.\(^2\)\(^-\)\(^2\)\(^1\) SOX3 is expressed at a low level in the gonad up to day 11.5, as is SRY.\(^2\)\(^3\) These patterns are compatible with competitive inhibition of SOX3 protein by SRY protein in binding to and regulating transcription of the SOX9 gene. In XX embryos, SOX3 protein would bind to SOX9 and inhibit its transcription. In XY embryos, SRY protein would compete with SOX3 binding and relieve this inhibition, permitting transcription of SOX9 and initiating testis differentiation.

An alternate candidate gene for a regulator of SOX9 is DAX1,\(^2\)\(^1\) thought to be responsible for the male-to-female sex reversal in XY individuals with a duplication of part of the short arm of the X chromosome.\(^3\)\(^0\) The pattern of DAX1 expression is similar to that of SRY\(^3\)\(^1\) and DAX1 has been suggested to interact with SRY in the testis-determining pathway. The dosage-sensitive nature of this action is reminiscent of many sex-determining systems throughout the animal kingdom and suggests that DAX1 dose may have been part of an ancestral mammalian sex-determining system. However, unlike SOX3, DAX1 is autosomal in marsupials,\(^3\)\(^2\) so was undoubtedly not a part of the ancestral mammalian X.\(^3\)\(^3\) DAX1 may therefore be a less attractive candidate than SOX3.

**PREDICTIONS OF THE HYPOTHESIS**

The hypothesis that the testis-determining action of SOX9 is regulated by SRY and SOX3 makes a number of testable predictions of the sexual phenotype of humans or other mammals with changes in the dosage or activity of any of these three genes. Table 1 presents the predicted phenotypes of XX and XY humans or mice with different types of mutation in SRY, SOX3, or SOX9. Of the twelve possible classes, the seven that have been observed all have phenotypes consistent with the predictions of the hypothesis.

**SRY**

Absence of SRY in normal XX individuals would lead to a female phenotype, as observed. Mutation or deficiency of SRY would produce male-to-female sex reversal as observed in XY (SRY\(^-\)) human females, and in XX female transgenic mice expressing low levels of Sry.\(^4\)\(^,\)\(^5\) Addition of SRY to an X or autosome by translocation would produce XX (SRY\(^+\)) males (as observed in most human XX males and in XX\(^3\)\(^0\) mice) and transgenesis would produce male mice.\(^5\) Overproduction or duplication (e.g., in XXY males) would have no effect on sex determination, as observed.\(^3\)\(^4\) Thus, all three predictions are upheld. The curious case of conditional SRY and SOX9 mutations transmitted from an apparently normal father to XY sex-reversed daughters\(^4\)\(^,\)\(^3\)\(^5\) could also be accounted for by an altered balance of SRY, SOX3 and SOX9 in individuals with different genetic backgrounds.

**SOX3**

There is scant evidence to assess the effects of mutation or deletion of SOX3. Mutation or deficiency of SOX3 should have no effect on male sex determination in XY embryos but could lead to XX(SRY\(^-\)) males if the genital ridge were populated largely by cells in which the X bearing the normal SOX3 allele were inactive. Deletion of SOX3 still permits testis development in XY children,\(^2\)\(^4\) as predicted, but there are conflicting results from Sox3 knockout experiments in mice. It would be particularly interesting to search for SOX3 mutations among XX(SRY\(^-\)) males, whose phenotype is particularly difficult to explain. Duplication or overproduction of SOX3 should not affect the female phenotype of XX embryos, but could produce XY(SRY\(^+\)) sex-reversed females. No duplications of this region of the human X are available,\(^3\)\(^6\) but the hypothesis could be tested by the phenotype of XY mice transgenic for additional copies of SOX3, as well as by searching for SOX3 mutations among human XY(SRY\(^+\)) females. Since SOX3 evidently has its major expression in the central nervous system, unrelated phenotypic effects of these mutations may be expected.

**SRY**

Deficiency of SOX9 would have no effect on the sexual phenotype of XX embryos but would lead to XY(SRY\(^+\)) females, as observed for many XY campomelic dysplasia patients.\(^2\)\(^2\) The variability in sexual phenotype among patients may be accounted for by differences in genetic background, since on this hypothesis, testis determination depends on a balance between SOX9, SOX3, and SRY expression. Duplication or overproduction of SOX9 would

### Table 1. Predictions of the Hypothesis*

<table>
<thead>
<tr>
<th>Mutation</th>
<th>SRY</th>
<th>SOX3</th>
<th>SOX9</th>
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<tbody>
<tr>
<td>XX SRY(^-) ovary</td>
<td>XX SRY(^-) testis</td>
<td>XX SRY(^-) ovary</td>
<td></td>
</tr>
<tr>
<td>NR(^a)</td>
<td>(SRM)(^b)</td>
<td>(SRM)(^b)</td>
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<tr>
<td>XY SRY(^-) ovary</td>
<td>XY SRY(^+) testis</td>
<td>XY SRY(^-) ovary</td>
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<tr>
<td>SRP(^a)</td>
<td>(NM)(^a)</td>
<td>(SRP)(^a)</td>
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<tr>
<td>XX SRY(^+) testis</td>
<td>XX SRY(^-) ovary</td>
<td>XX SRY(^-) testis</td>
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<td>SRP(^a)</td>
<td>(NM)(^a)</td>
<td>(SRP)(^a)</td>
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<td>XY SRY(^+) ovary</td>
<td>XY SRY(^+) testis</td>
<td>XY SRY(^+) testis</td>
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</tr>
<tr>
<td>NM(^a)</td>
<td>(SRF)(^b)</td>
<td>(NM)(^c)</td>
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*Gonadal phenotypes (testis or ovary) in normal (N) or sex-reversed (S) male (M) or female (F) phenotypes predicted by the hypothesis for individuals with deficiency/mutation (\(-\)) or duplication/overproduction (+) of SRY, SOX3, and SOX9.

\(^a\)There are seven classes confirmed by observation.

\(^b\)Five classes remain to be tested.
have no effect on the sex of XY males, but could produce XX(SRY-) males. These predictions could be tested by searching for SOX9 mutations among XY(SRY+) females and XX(SRY-) males, and ascertaining the sexual phenotypes of mice transgenetic for extra copies of SOX9.

Thus, this hypothesis offers three ways of accounting for XY females. XY(SRY-) females are well documented but form a minority of this class.4 XY(SRY+) females could result from a duplication or overproduction of SOX3, as well as a deficiency of SOX9. More significantly, the hypothesis provides two alternative explanations for XX(SRY-) males, which could arise by deletion or mutation of SOX3, or by duplication or overproduction of SOX9.

The hypothesis can also explain instances of ovary to testis transdifferentiation in the absence of SRY.15 For instance, the formation of testis tissue in foetal ovaries in culture, or grafted to the kidney capsule of adult XY or XX mice would be easy to account for if SOX3 were present in culture or in these ectopic sites at a concentration too low to inhibit SOX9. This seems likely, given that SOX3 expression is confined to the CNS of the embryo. It should be possible to measure relative rates of SOX3 and SOX9 transcription, at least in the culture systems.

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,37 may contain an overexpressed or duplicated SOX3. This could readily be tested. In the complete absence of SRY in mole vole species with no Y chromosome,38 SOX9 could be regulated by a completely novel gene, with or without the involvement of SOX3 or another SOX gene.

**EVOLUTION OF THE SEX-DETERMINING ROLE OF SRY IN MAMMALS**

Its male gonad-specific expression in chicken as well as mouse suggests that SOX9 was part of an ancestral vertebrate sex-determination pathway that predated the divergence of lineage-specific XX/XY or ZZ/ZW sex-determining systems more than 350 million years ago. In birds and other nonmammalian vertebrates in which human X-linked genes are autosomal and there is no sex-specific SRY, SOX9 may be regulated by an entirely different control pathway, with or without the involvement of SOX3 or another SOX gene on the Z or W chromosome. However, the downstream steps of gonad differentiation are likely to be identical.

The involvement of dosage differences in sex determination is a recurring theme among diverse animal groups.39 The mammalian sex-determining pathway might therefore have evolved through an intermediate stage in which dosage of SOX3, rather than inhibition by SRY, regulated SOX9 and controlled testis determination (Fig. 2).

In an ancestral mammal or mammal-like reptile with undifferentiated or partially differentiated X and Y chromosomes and no X inactivation, an autosomal or pseudoautosomal SOX3 may initially have interacted with SOX9 to accomplish gonad development in both sexes, under the control of some ancestral sex-specific signal. If a new, inactive SOX3 allele (SOX3null) arose, a dosage difference between heterozygotes and homozygous normal would be set up which could differentially regulate SOX9. Homozygotes (with two active SOX3 alleles, genotype SOX3 SOX3) would make sufficient SOX3 product to repress the testis-determining action of SOX9, and would therefore be female. Heterozygotes (with two active SOX3 alleles, genotype SOX3 SOX3null) would have insufficient SOX3 activity to repress SOX9 and would therefore develop testis and be male. I suggest that this differentiation of SOX3 alleles allowed SOX3 to take over a SOX9 regulating function from the ancestral sex-specific signal. This could have been either
the initiating event of X and Y chromosome differentiation, or the result of the continuing degradation of a proto-Y chromosome that bore the ancestral regulator.

An improvement on this dosage system could then have developed with the evolution of the inactive SOX3\textsuperscript{null} allele on the proto-Y chromosome into a truncated competitive inhibitor. Thus the degraded SOX3\textsuperscript{null} allele became SRY, an HMG box-containing minigene that bound to the same target sequence as SOX3 and blocked its inhibiting action on SOX9. Active inhibition of SOX3 by SRY would lead to much more robust control than the older SOX3 dosage-regulated system, and would be selected. Once inhibition of SOX3 by the Y-borne SRY was established, a dosage difference between male and female became redundant, and SOX3, like other genes on the X whose Y partners were lost or acquired a new function, was recruited into the X inactivation system.

Evidence for the involvement of autosomal and X-linked genes in several sex-reversal syndromes in humans and other mammals suggests that the pathway is more complex than considered here and involves genes that have functions other than sex determination. For instance, SOX9 controls the expression of collagen genes in developing cartilage,\textsuperscript{10,41} and its deficiency leads to campomelic dysplasia.\textsuperscript{18,19} SOX3 is expressed early in the CNS\textsuperscript{24} and presumably is involved in CNS differentiation. It is quite conceivable that SRY also retains additional functions in the mammals in which it has a wide expression profile. DAX1 may be involved in ovarian as well as adrenal development and may interact with SRY or SOX9.\textsuperscript{21,31} and SRY may interact with the mullerian inhibiting substance (MIS) gene.\textsuperscript{42} This complexity and pleiomorphism should not be surprising, by analogy with the pathways of Drosophila and Caenorhabditis sex determination. Indeed, it would be surprising if mammal sex determination were as simple as we naively supposed a decade ago.

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REFERENCES


