Sry, more than testis determination?

Monte E. Turner, Daniel Ely, Jeremy Prokop, and Amy Milsted

Department of Biology, The University of Akron, Akron, Ohio

Submitted 24 September 2010; accepted in final form 1 June 2011

Turner ME, Ely D, Prokop J, Milsted A. Sry, more than testis determination? Am J Physiol Regul Integr Comp Physiol 301: R561–R571, 2011. First published June 15, 2011; doi:10.1152/ajpregu.00645.2010.—The Sry locus on the mammalian Y chromosome is the developmental switch responsible for testis determination. Inconsistent with this important function, the Sry locus is transcribed in adult males at times and in tissues not involved with testis determination. Sry is expressed in multiple tissues of the peripheral and central nervous system. Sry is derived from Sox3 and is similar to other SOXB family loci. The SOXB loci are responsible for nervous system development. Sry has been demonstrated to modulate the catecholamine pathway, so it should have functional consequences in the central and peripheral nervous system. The nervous system expression and potential function are consistent with Sry as a SOXB family member. In mammals, Sox3 is X-linked and undergoes dosage compensation in females. The expression of Sry in adult males allows for a type of sexual differentiation independent of circulating gonadal hormones. A quantitative difference in Sox3 plus Sry expression in males vs. females could drive changes in the transcriptome of these cells, differentiating male and female cells. Sry expression and its transcriptional effects should be considered when investigating sexual dimorphic phenotypes.

SRY; sexual dimorphism; SOX; nervous system

IT IS WELL UNDERSTOOD THAT many genes and gene products have pleiotropic effects, yet for some loci, a known essential function hides this reality. A newly derived function may distract attention from ancestral functions. In physiology, hormones have many more functions than that of their original functional discovery. The Sry locus on the Y chromosome is responsible for testis determination in placental mammals. The essential biological importance of sex determination and difficulties in describing the mechanism have overshadowed the potential of Sry functions not related to testis determination. In addition, the prevailing ideas of mammalian sex determination rely on the ovariies/testes producing estrogens/androgens for the female/male phenotypes. Mammalian sex is determined at the autosomal in most mammals, and Sox3 is X-linked. It is believed that Sry originated from a mutation of Sox3 on the then autosomal primordial mammalian X chromosome, leading to the advent of the mammalian Y chromosome (81). The amount of HMG amino acid sequence homology between SRY and other SOX family members supports this theory, with Sox3 having the highest amino acid sequence conservation. Some regions outside of the HMG box and hinge regions have been suggested to play a role in SRY-specific function (79). There is a slight amount of amino acid sequence conservation across SRY in multiple species in the bridge domain. This bridge domain has been shown to interact with KRAB-O (64), and there is no indication that this conserved KRAB-O domain is involved in testis determination. The conservation in the SRY-specific bridge domain could be involved in long-term transcriptional regulation. Only members of the SOXB group have
several amino acids conserved in this domain and may explain similar functions of SRY and SOXB members. A recent study by Sato et al. (81) reanalyzed the sequence of the human Sry gene by searching for homologous DNA sequences. They concluded that the gene evolved as a hybrid resulting from insertions of a part of the DGCR8 gene, which encodes Di-George syndrome critical region 8 and is involved in biogenesis of microRNA (34), into a region 5′ of the HMG box of an ancestral Sox3 gene.

Sry as a Y Chromosome Locus

The constraints on mutations and evolution of Sry are different from an autosomal locus. Sry is located on the mammalian Y chromosome and is thus constrained by this location. The Y acts as homologous chromosome with the X chromosome in meiosis but does not recombine except at specific regions (pseudoautosomal region). This results in the majority of the Y chromosome being inherited as a single locus. The classic view of the Y chromosome is that of a chromosome that accumulates mutations with selection eliminating Y chromosomes with mutations that have lost essential functions. A modern version of the Y chromosome includes the observation that certain regions of the Y chromosome contain almost identical repeats that have high frequencies of gene conversion that can “fix” mutations that occur (77).

The human Y chromosome is composed of three types of sequences: ampliconic, X-transposed, and X-degenerate (87). The ampliconic regions contain large repeat units in which gene conversion and homologous recombination occur within the repeat regions (74). The X-degenerate and X-transposed regions have origins from ancestral autosomes (X-degenerate) and more recent transpositions from the X chromosome (X-transposed) (48). Both the X-degenerate and X-transposed regions contain primarily single-copy genes, which should evolve like the classic view of the Y chromosome. The Sry locus is within the X-degenerate region of the human Y chromosome. Structural mutations in individual Y chromosomes may have moved the Sry locus into different regions of the chromosomes in other mammalian species. The location of Sry in different regions of the Y chromosome may affect the evolution of the Sry locus in these species.

Because Sry is located in the X-degenerate region of the human Y chromosome, any mutation not affecting function of the locus should accumulate among human Y chromosomes. We might expect the accumulation of synonymous mutations in the coding region, nonsynonymous mutations outside of the HMG-box region not affecting DNA binding, and mutations in the flanking regions not involved in transcriptional control or stability. The available data demonstrate that the X-degenerate and X-transposed regions have reduced levels of genetic diversity compared with autosomal regions (78). Consistent with this observation, the effective population size of a Y chromosome is only ¼ that of an autosomal chromosome, thus reducing the opportunity for mutations.

In a study of the coding regions of genes from 105 human Y chromosomes, selected to examine the range of human Y chromosomes, nucleotide diversity was significantly lower for nonsynonymous sites than synonymous sites, pseudogenes, or introns, and this is consistent with natural selection maintaining these gene sequences. It is possible that Sry is the force driving this purifying selection as any mutation that eliminates function in an essential developmental switch will be immediately lost from the population. The sequence variation in Sry is among the lowest on the Y chromosome, with only one substitution (synonymous) found in the coding region of Sry (78).

To examine the variation outside of the coding region, we can compare the Sry locus in the male personal genomes that have been published and available. The genomes of KB1 (83), NB1 (83), ABT (83), George Church (16), Henry Louis Gates Sr. (16), J. Craig Venter (50), James Watson (97), YRI (17), Han Chinese Individual (94), Seong-Jin Kim (1), Anonymous Korean individual (42), Stephen Quake (70), and the 4,000 year old Saqqaq (72) were viewed using the PSU Genome Browser (http://main.genome-browser.bx.psu.edu/). Only Seong-Jin Kim genome has an entire nucleotide polymorphism (SNP; synonymous) within the coding region of Sry (chrY: 2,714,896–2,715,792, numbering from NCBI36/hg18). Looking at 4 kbp on each side flanking the Sry-coding sequence (chrY: 2,710,859–2,719,828) reveals only four additional SNPs, but none shared among multiple sequences. Of interest is that the Saqqaq sequence reveals no SNPs over this stretch of the Y chromosome, suggesting that the sequence in this region has remained stable over the past 4,000 years (85). The Sry flanking region shows the same reduction in variation consistent with results using only the coding region (78).

Other than the comparison using these genomes, there is no survey of normal variation in the Sry locus. A study of gonadal dysgenesis can only identify mutations that disrupt testis determination, but these are lost from the population because the phenotype is sterile (31). These results from a comparison of different human Sry sequences, coding region (78) and flanking regions, and coding region of both modern and ancient genomes (Saqqaq), indicate that the gene does not have the necessary polymorphism levels to answer either questions of additional function or to determine sequences necessary for testis determination using conserved regions because the whole region seems to be highly conserved.

Multiple Copies of Sry on a Single Y Chromosome

In some rodent species, Sry has multiple copies on a single Y chromosome (60). Multiple copies have been demonstrated in family Muridae, including African rodents (53, 60), voles (6), and rats (90). These are true duplicate loci with independent control and coding regions. The exact spatial relationship between any of these loci is unknown, as none of these Y chromosomes have been sequenced. In the rat, indirect evidence indicates the loci are not close to each other, as sequenced BACs only contain a single copy. The finding of copies in many related species indicates these copies are either very old or their location on the Y chromosome causes duplications to occur at an increased rate. The presence of many copies in these rodents does not seem to have any obvious deleterious effects. In voles, the multiple copies are obviously not functional due to nonsense/frameshift mutations in the coding region (7), but for these mutations to have occurred and accumulated, the duplicate Sry loci must be very old.

In other examples, the coding regions do not contain mutations that would lead to nonfunctional proteins or evidence of Sry pseudogenes (53, 90, 91). Expression patterns have only
been examined in *Rattus norvegicus* strains, and the multiple copies on the rat Y chromosome show divergent expression patterns (90), consistent with either gain or loss of transcriptional components. In addition to transcriptional divergence these *Rattus norvegicus* *Sry* loci have slightly different protein phenotypes and have potential divergent effects in the adult male rat.

The occurrence of multiple *Sry* copies is probably a result of the repeat organization of the mammalian Y chromosome leading to many species-specific duplications and deletions. Multiple *Sry* copies have been identified in human males exposed to high background radiation but not in normal control males (68, 69). The question of how common are multiple copies of *Sry* on a single Y chromosome remains largely unanswered because experiments to identify whether multiple copies exist have either not been tested or not enough human Y chromosomes have been sequenced to characterize a population.

**Sry as a Member of the SOXB Family**

In the human and mouse genomes there are 20 members of the SOX gene family (82), many of which have known roles in disease etiology. Focusing on the SOXB family (*Sox1*, *Sox2*, and *Sox3*), which is most similar to *Sry*, all members are important for stem-cell maintenance in the central nervous system (95), and *Sox2* and *Sox3* are important in pituitary development (2). All three are coexpressed in both avian and murine neural progenitor cells and the adult brain (8, 102). Null mutations of *Sox1*, *Sox2*, and *Sox3* have different neuronal phenotypes, indicating differentiation of function (18, 28, 76). Lost in most discussions of *Sry* and testis determination is the idea that *Sry* was derived from *Sox3*, and testis determination is a new derived function rather than the ancestral central nervous system function (33).

*Sox3* is expressed in neural progenitor cells, and both *Sox3* and *Sry* are expressed in adult testis (96). Consistent with *Sox3* derivation, *Sry* expression in the adult testis is an ancestral condition, not derived. *Sox3* deletions in mice have abnormal development of diencephalon and anterior pituitary gland and lack differentiated spermatogonia (73). *Sox3* has been speculated to activate *Sox9* and induce sex reversal in XX transgenic mice, providing a similar outcome as *Sry* expression (4, 51, 92). If *Sry* and *Sox3* can behave similarly in testis development, can they also behave similarly in the brain?

*Sry* is expressed in the substantia nigra of tyrosine hydroxylase (*Th*)-expressing cells (15) and has been demonstrated to increase transcription of the *Th* promoter (56). Tyrosine hydroxylase is the rate-limiting enzyme for norepinephrine and dopamine synthesis, suggesting that *Sry* has a role in male-specific brain development. *Sry* transcripts have been found in an ever expanding number of tissues (Table 1), so that understanding the mechanisms by which *Sry* works becomes increasingly important. The expression pattern of *Sry* in adult males is better explained if *Sry* is examined as a SOXB-derived locus rather than primarily as a testis determination locus. Does *Sry* function similarly to other members of the SOXB family or has it specialized through alterations in preferred DNA binding sequences or protein binding partners?

Since *Sox3* is an X-linked locus, it is subject to X inactivation. Therefore, mammalian females have *Sox3* from one X chromosome expression, while males have *SOX3* plus *SRY* in tissues, where *Sry* is expressed. Any quantitative relationships would be enhanced in males over females. This difference is independent of steroid hormones. Expression of *Sry* in males adds a level of cellular sex determination and differentiation independent of gonads and hormones. The interaction of SRY with the androgen receptor could further potentiate these affects in male cells vs. female cells (104). This mechanism is potentially present in the brain and nervous system, both during development and in adult males due to the expression of *Sry* in these tissues and cells.

**Sry Expression**

Table 1 lists published results and unpublished results from our laboratory of *Sry* expression at times and in tissues not related to testis determination. The number of tissues where expression has been identified and the conservation of expression in many species are not the expectation or prediction for a gene involved only in testis determination. Any mutation eliminating unnecessary expression would be advantageous, and the expectation would be random loss of expression in a single species and decreased expression phylogenetically over evolutionary time in a Muller’s ratchet type of expectation (59).

In SHR and WKY rat strains, we have examined the expression in numerous tissues of the six or seven duplicated copies of the *Sry* locus (Table 1) (90). Multiple *Sry* loci are expressed in kidney (23, 90), adrenal gland, brain, and other tissues in young and adult male rats (Table 1) (56, 90, 91). We have used *Sry*-specific primers to amplify cDNA from many rat tissues (Table 1) and find the presence of *Sry* in tissues tested and no amplification in no-RT controls or females. Many of these rat *Sry* loci are expressed, in patterns that are tissue- and locus-dependent. Again, the pattern is one expected with function, rather than random loss, or the same expression pattern, for all duplicated loci.

*Sry* expression is found in many different tissues and times in addition to the genital ridge at the time of testis determination. For instance, Dewing et al. (15) identified *Sry* expression in situ in the rat brain using an antisense *Sry* probe and SRY protein with a anti-SRY antibody. Modi et al. (58) identified *Sry* mRNA in situ in human adult male testis with an antisense probe. These results are significant because *Sry* expression is not seen in expressed sequence tag (EST) libraries of these tissues. In fact, *Sry* expression is missing from all EST library sequences. If there is enough expression for in situ identification, one would expect multiple EST sequences for *Sry* from these and other tissues. This lack of ESTs for *Sry* is not species specific but is deficient in all libraries, even from species and tissues where *Sry* expression has been identified from methods that require a much larger number of transcripts. The only conserved sequence among these species is the HMG box, hinge region, and bridge domain of the gene; thus, these regions must have a sequence or secondary structure that inhibits cloning under standard conditions. Because we are able to amplify the entire rat *Sry* sequences in cDNA made from mRNA, the lack of *Sry* in EST libraries is probably a result of inefficient cloning rather than in the process of creating cDNA.
From our experience with rat Sry sequences, it is obvious that the Sry sequence does not clone with the same efficiency as other sequences. In fact, not all of the Sry loci from a single rat Y chromosome clone equally well. Using primers spanning the rat Sry coding sequences, the Sry2 amplicon is 39 bp shorter than the other sequences and can be seen as a separate band with electrophoresis (91). Using PCR TOPO TA cloning kit to clone the amplicons, we sequenced more than 100 clones and found that no clones contained the Sry2 sequence, even though it would be expected that either 1/6 or 1/7 of the clones would be Sry2. The Sry2 sequence was eventually determined from Sry2-specific primers and sequencing of that PCR product (91).

### Sox Loci in the Nervous System

Since the initial discovery of Sry 20 years ago and subsequently the SOX loci, numerous studies have shown a strong role for SOX transcription factors in the nervous system of vertebrates and invertebrates. Invertebrates, including Drosophila (61), Amphioxus (38), and the Ptychodera flave (89) have Sox expression in the nervous system. In all three of these species, homology of the Sox proteins involved most closely matches that of the SOXB1 group. All SOXB1 genes have been shown to be expressed in the central nervous system (CNS) of vertebrates (12). The most studied of the SOXB1 members is Sox2, which is important for nerve cell formation and development.

<p>| Table 1. Sry expression in mammalian tissues and method of detection |</p>
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Source (A, F)</th>
<th>Detection Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal gland</td>
<td>rat (A)</td>
<td>real-time RT-PCR, gel-based</td>
<td>56, 90, 91, unpublished data</td>
</tr>
<tr>
<td>Brain</td>
<td>human (F)</td>
<td>gel-based RT-PCR</td>
<td>11</td>
</tr>
<tr>
<td>Amygdala</td>
<td>rat (A)</td>
<td>real-time RT-PCR</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Brainstem</td>
<td>human (F)</td>
<td>gel-based RT-PCR</td>
<td>11</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>rat (A)</td>
<td>gel-based and real-time RT-PCR</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Cortex</td>
<td>rat (A)</td>
<td>in situ hybridization</td>
<td>15</td>
</tr>
<tr>
<td>Cortex</td>
<td>mouse (A)</td>
<td>gel-based RT-PCR</td>
<td>55</td>
</tr>
<tr>
<td>Diencephalon</td>
<td>mouse (A)</td>
<td>gel-based RT-PCR</td>
<td>55.1</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>rat (A)</td>
<td>real-time RT-PCR</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Locus coeruleus</td>
<td>rat (A)</td>
<td>gel-based PCR</td>
<td>56</td>
</tr>
<tr>
<td>Medial mammillary bodies</td>
<td>rat (A)</td>
<td>in situ hybridization</td>
<td>15</td>
</tr>
<tr>
<td>Midbrain</td>
<td>mouse (A)</td>
<td>gel-based RT-PCR</td>
<td>55</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>rat (A)</td>
<td>gel-based PCR</td>
<td>56</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>rat (A)</td>
<td>in situ hybridization (ISH)</td>
<td>15</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>rat (A)</td>
<td>immunohistochemistry (protein)</td>
<td>15</td>
</tr>
<tr>
<td>Ventral tegmental area</td>
<td>rat (A)</td>
<td>gel-based PCR</td>
<td>56</td>
</tr>
<tr>
<td>Celiac ganglion</td>
<td>rat (A)</td>
<td>real-time RT-PCR</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Cultured cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neonatal astrocytes</td>
<td>rat</td>
<td>real-time RT-PCR</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Hep G2</td>
<td>human</td>
<td>gel-based RT-PCR</td>
<td>11</td>
</tr>
<tr>
<td>NTERA-2 cl.D1</td>
<td></td>
<td>gel-based PCR</td>
<td>11</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aorta</td>
<td>rat (A)</td>
<td>real-time RT-PCR</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Atrium</td>
<td>rat (A)</td>
<td>real-time RT-PCR</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Heart</td>
<td>human (A)</td>
<td>gel-based RT-PCR</td>
<td>11</td>
</tr>
<tr>
<td>Heart</td>
<td>human (F)</td>
<td>gel-based RT-PCR</td>
<td>11</td>
</tr>
<tr>
<td>Ventricle</td>
<td>rat (A)</td>
<td>real-time RT-PCR</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Kidney</td>
<td>rat (A)</td>
<td>real-time RT-PCR</td>
<td>91, unpublished data</td>
</tr>
<tr>
<td>Kidney</td>
<td>human (A)</td>
<td>gel-based RT-PCR</td>
<td>11</td>
</tr>
<tr>
<td>Liver</td>
<td>rat (A)</td>
<td>real-time RT-PCR</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Liver</td>
<td>human (A)</td>
<td>gel-based RT-PCR</td>
<td>11</td>
</tr>
<tr>
<td>Liver</td>
<td>human (F)</td>
<td>gel-based RT-PCR</td>
<td>11</td>
</tr>
<tr>
<td>Pancreas</td>
<td>human (F)</td>
<td>gel-based RT-PCR</td>
<td>11</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>rat (A)</td>
<td>real-time RT-PCR</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Small intestine</td>
<td>human (F)</td>
<td>gel-based RT-PCR</td>
<td>11</td>
</tr>
<tr>
<td>Spleen</td>
<td>human (F)</td>
<td>gel-based RT-PCR</td>
<td>11</td>
</tr>
<tr>
<td>Superior cervical ganglia</td>
<td>rat (A)</td>
<td>real-time RT-PCR</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Thymus</td>
<td>human (F)</td>
<td>gel-based RT-PCR</td>
<td>11</td>
</tr>
<tr>
<td>Testis</td>
<td>rat (A)</td>
<td>real-time RT-PCR</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Testis</td>
<td>bovine (A)</td>
<td>Northern blot</td>
<td>13</td>
</tr>
<tr>
<td>Testis</td>
<td>mouse (A)</td>
<td>Northern blot, gel-based RT-PCR</td>
<td>46</td>
</tr>
<tr>
<td>Testis</td>
<td>mouse (A)</td>
<td>Northern; nuclease protection; ISH</td>
<td>108</td>
</tr>
<tr>
<td>Testis</td>
<td>human (A)</td>
<td>Northern blot</td>
<td>86</td>
</tr>
<tr>
<td>Testis</td>
<td>human (A)</td>
<td>Northern; gel-based PCR</td>
<td>11</td>
</tr>
<tr>
<td>Gonadal ridge</td>
<td>mouse (F)</td>
<td>Northern; ISH; gel-based RT-PCR</td>
<td>46</td>
</tr>
<tr>
<td>Preimplantation embryo</td>
<td>mouse (F)</td>
<td>Gel-based RT-PCR</td>
<td>107</td>
</tr>
<tr>
<td>Male embryo</td>
<td>porcine (F)</td>
<td>Gel-based RT-PCR</td>
<td>14</td>
</tr>
<tr>
<td>Preimplantation embryo</td>
<td>human (F)</td>
<td>Gel-based RT-PCR</td>
<td>29</td>
</tr>
</tbody>
</table>

Unpublished data are from the spontaneously hypertensive rat and Wistar-Kyoto strains from our laboratory. Source: A, adult; F, fetal or embryonic.
in the inner ear (63), parenchymal astroglia cell formation (45), maintenance of neural stem cells (9), and neurogenesis in the inner ear (63), parenchymal astroglia cell formation (45), and regulation of nicotinic ACh receptors (52). SOXC genes (Sox4) development (47), oligodendrocyte development (88), and regulation of nicotinic ACh receptors (52). SOXC genes (Sox4 and Sox11) are expressed in the immature neurons of neural stem cells in mice (66). Because SRY shares a HMG box, and thus similar DNA binding sites, with all other SOX proteins, SRY, when expressed could participate in similar roles in nervous system development and function in the adult male nervous system.

Sry, the Peripheral Nervous System and Neuroendocrine Regulation

Our research demonstrated that Sry has a role in the peripheral nervous system, specifically the sympathetic nervous system. TH is the rate-limiting enzyme for catecholamine biosynthesis both in the brain and periphery. Transient cotransfection assays in PC12 cells with Sry1 and pHt/Luc(-272/+27) demonstrated that the product of the Sry1 gene increases expression of the luciferase gene under control of the Th promoter (56). When an AP-1 mutated site in the Th promoter was used, luciferase activity decreased by ~75% compared with the control Th promoter. This suggested that the actions of Sry in regulating TH transcription are due to interactions at the AP-1 site (75%) and the direct effects of Sry (25%). The impact of SRY on AP-1 transcription factors is not unexpected, given the previously identified targets of SRY (30). The impact of Sry on the sympathetic nervous system has a diverse potential on multiple phenotypes in humans and other mammals, such as the fight-flight response, blood pressure, and kidney function.

The sympathetic nervous system is not the only potential target of Sry in the peripheral nervous system. The renin-angiotensin system (RAS) is also a target of Sry regulation (57). Recently, we have shown that the human SRY protein also regulates rat angiotensin-converting enzyme (Ace), Ace2, renin (Ren), and angiotensinogen (Agt) promoter activity (Fig. 1), suggesting that Sry regulation of target genes is similar and conserved across species. Our modeling studies comparing human and rat SRY proteins indicate close structural conservation across these species (Fig. 2). The effects of rat Sry on the rat RAS were investigated by cloning the coding sequences of Sry1, Sry2, and Sry3 genes into expression constructs and cotransfecting each with the promoters of the rat RAS genes Agt, Ren, Ace, and Ace2 in luciferase reporter plasmids. All Sry effectors (proteins) increased activity of the promoters of Agt, Ren, and Ace and decreased activity of Ace2. The largest effects were seen with Sry3, the locus that activated the RAS and raised blood pressure after delivery to a rat kidney (23). Because regulatory control regions in promoters of these genes are generally conserved across species, we would predict that the human RAS gene promoters will also respond to human SRY effectors.

The RAS consists of both the classical form in the circulating system and local tissue RASs. In addition to the main participants in the classical pathway (Agt, Ren, and Ace), alternate pathways have been described, and enzymes such as angiotensin I-converting enzyme (ACE2) are known to play important roles in RAS function (25, 27, 32, 40, 65). To have a significant effect on the RAS, an effective regulator of expression of RAS genes would modulate more than one gene. The more genes in the cascade that are coordinately modulated by the transcriptional regulator, the larger the overall effect can be. This is the case with SRY; SRY increases activity of the promoters of Agt, Ren, and Ace, and it decreases Ace2 promoter activity. The effect of SRY on the RAS is essentially amplified by up-regulating several genes, including the substrate angiotensinogen, through the processing enzymes renin and ACE, while simultaneously down-regulating ACE2. Taken together, these would favor the increased generation of ANG II.

Hypertension: Effect of Sry on the Peripheral Nervous System

In the spontaneously hypertensive rat (SHR) model of human hypertension, the Y chromosome and Sry account for a significant portion of the blood pressure increase in males over normotensive rat strains (21). We recently published a summary of our Y chromosome, Sry, and hypertension results in Steroids (24) and only a brief summary is reported here.

Our initial blood pressure studies showed that Wistar-Kyoto (WKY) males with the SHR Y chromosome had elevated blood pressure compared with WKY males with a WKY Y chromosome (19). This was the first evidence that a locus on the SHR Y chromosome influenced blood pressure. Further studies examining the mechanism of this blood pressure increase demonstrated that many indices of sympathetic nerve activity (SNS) activity, such as adrenal gland chromogranin A and TH, were elevated (20). Pharmacological reduction of plasma NE reduced the Y chromosome blood pressure effect (99). Next, our focus switched to identifying the Y chromosome locus that
influenced blood pressure. Sry was a candidate locus, and we found multiple copies of Sry in WKY and SHR; however, SHR had at least one copy of Sry that was different (90).

When Sry1 was given exogenously to the kidney or adrenal gland of normotensive WKY males, BP and SNS markers were elevated (22, 23). When another copy, Sry2 was delivered to the WKY kidney, BP was not affected (23). However, when a third copy, Sry3, was delivered to the kidney, the RAS was activated along with sodium retention and BP elevation, but SNS markers were not affected (24). The Sry3 locus in SHR may synergistically act with Sry1 to elevate BP pressure through an interaction of the SNS and RAS. We speculate that the differential function of Sry1 vs. Sry3 may be due to the amino acid substitution in Sry3. These results are consistent with an increased SNS activity mechanism due to Sry1, and increased tissue renin angiotensin system activity due to Sry3.

The functions of the other copies of the Sry gene complex on the Y chromosome remain to be determined. Although the phenotype originally described and examined was hypertension, the underlying mechanism is the effect of Sry on the sympathetic nervous system and RAS.

Consistent with a more global response of Sry on Th is the report that a mutation in a SRY binding site in human Th has a significant correlation with human blood pressure (106). Sry also binds and modifies transcription related to a known SNP in the human chromagranin B locus, consistent with a human SRY effect on the sympathetic nervous system (105). Because the human RAS gene promoters also contain conserved SRY and AP-1 binding sites, they should also respond to SRY. We suggest that the effects we describe with Sry on RAS gene regulation are reflected in the effects of human Sry on human BP, as well as on numerous other systems and proteins not yet identified. These results suggest that Sry regulation of target genes is very similar across species.

Note that these are male effects in neuroendocrine regulation and the peripheral nervous system that are not related to androgens or other circulating factors, but to the effects of Sry expression in these cells. Female cells would not be expected to have these responses.

**Sry in the Central Nervous System**

Sry also is present and expressed in the central nervous system of both humans and rodents. Monoamine oxidase (MAO) is a degradative enzyme for a number of biogenic amines, which are involved both in peripheral, as well as central nervous system function, such as serotonin, norepinephrine, and dopamine. Wu et al. (103) have shown the presence of a functional binding site for SRY in the MAO A core promoter both in vitro and in vivo. SRY activates both MAO A-promoter and catalytic activities in a human male neuroblastoma BE(2)C cell line. This finding has important biological and medical implications because the effect of SRY on MAO A expression could be part of a sexually dimorphic program in neural structure and physiology. In addition, SRY effects on MAO A may explain sex differences in cognition, behavior, and psychiatric disorders that involve the biogenic amines. Specifically, Sry is known to be expressed in the midbrain of the rat (15), the hypothalamus and midbrain of the mouse (54), and the hypothalamus and frontal and temporal cortex of the human brain (51). Expression in the rat substantia nigra is associated with agonistic behavior (15). We found Sry
expression in most rat brain areas examined (Table 1). The following data support the concept that Sry has CNS effects that may be important for understanding neurotransmitter regulation and nervous system phenotypes.

In the brain of a normotensive WKY rat male, we isolated eight different brain regions, and for each region, mRNA was isolated, and primers specific for different Sry loci were used to determine the expression of the different rat Sry loci in the mRNA pool (90). There were expression differences in the different brain regions. The proportion of Sry1 varies according to brain region, varying from about 10% to more than 50% of total Sry transcripts, while the proportion of the Sry2 varies from 45% to 90% of total Sry transcripts. Sry1 was transfected into the hippocampus of a SHR female, and after 5 days, TH was increased more than 100% compared with vector controls (Fig. 3).

Sry had not been thoroughly investigated with regard to its role in the serotonergic system, but it would not be unusual for transcription factors like Sry to play a role in development of 5-HT neurons. Tryptophan hydroxylase (Tph) is the rate-limiting enzyme for serotonin biosynthesis. When we analyzed the promoters of the mouse and human Tph genes with TRANSFAC (101), we found multiple potential SRY consensus binding sites. This suggested that SRY has the potential to regulate the rat Tph gene by a mechanism similar to that we described for the tyrosine hydroxylase (Th) gene (56). We then demonstrated in WKY males that if we added exogenous Sry1 to the amygdala, brain serotonin content decreased. Figure 4 shows that exogenous Sry delivered to the amygdala caused a significant reduction in serotonin content in the amygdala compared with controls (23% reduction, \( P < 0.05, n = 6\) group).

Overview of Sry Expression in Adult Males

The acknowledged primary function of Sry is testis determination but evolutionarily, this is a derived function. The Sry locus was derived from Sox3, which has functions in both the brain and nervous system. The divergence of Sry into testis determination did not mean all ancestral Sox3 function was lost. In fact, it may have required no loss of function. We see Sry expressed in many brain and nervous system tissues, and with the focus on testis determination, we examine these

Tyrosine Hydroxylase Activity

![Tyrosine Hydroxylase Activity Graph]

Fig. 3. Transfection of Sry1 to WKY female hippocampus increased tyrosine hydroxylase activity more than 100% compared with control vector (means ± SE, \( *P < 0.05 \)).

newly acquired expression or function when, in fact, they are remnants of the original function of Sry, as a derived Sox3 locus.

Sry is a transcription factor with a consensus binding site the same as other SOX loci. In any tissue where Sry is expressed, the transcriptome of that cell would be modified. Is this modification considered function? This modification does mean that male cells and female cells do not have the same transcriptome and this is not only the result of different sex hormones but includes the effects of Sry expression itself on gene regulation. Consistent with a nontestis determination function for Sry is the fact that Fra-1 and Fra-2 are reported Sry targets (30), which are not involved in testis determination but are components of the AP-1 transcription factor. Sry expression would increase AP-1 and hence any pathway controlled by AP-1. This model through AP-1 is consistent with the results of Sry on the Th promoter in rats (56). In a recent study examining the effect of human Sry using proteomics and transcriptome approaches, NT2/D1 cells from human testicular embryonal cell carcinoma were stably transfected with Sry (80). Two different lines were studied, SRY1 and SRY2, both of which overexpressed SRY. Using two-dimensional gels followed by mass spectrometry analysis to compare protein profiles in SRY-overexpressing cell lines to controls showed that SRY downregulated many chaperone proteins and upregulated laminin, which plays a role in Sertoli cell differentiation. Comparative analysis of the transcriptomes of the cell lines by microarray analysis using Affymetrix GeneChips showed that SRY upregulated many zinc finger proteins and downregulated cellular growth factors. Cell proliferation and cell cycle analysis indicated that SRY overproduction arrested cell cycle progression and inhibited cell proliferation (80).

Perspectives and Significance: Potential Impacts of Sry Expression and Function and Unanswered Questions

Whether these effects summarized here are restricted to the organisms in which they have been identified or are more widespread is not known, primarily because the experiments either have not been attempted or are not informative because of reduced genetic variation or the difficulty of separating sex determination and sex hormones from Sry effects. In a mouse
model, transgenic Sry may allow some testing, but confounding effects, in addition to the effects of other sex determination loci, include the amount of control region included in the transgene and expression and epigenetic effects of moving Sry from heterochromatin to euchromatin. Sry expression is conserved in many adult male mammalian tissues; the transcriptional effect of this expression on the cellular transcriptome needs to be established using current techniques like those of Sato et al. (80).

We raise further questions related to nontestis-determining actions and potential clinical relevance of Sry expression.

- **Sry** is expressed in the adult testis in only certain cell types (11). How does this effect the adult testis?
- There are high levels of Sry expression in early mammalian blastula (107). Are XX and XY cell lineages beginning to differ at this early stage or is it a male compensation for the fact that X-inactivation has not yet occurred?
- Is there increased expression of a related Sox locus in females that compensates for Sry expression in males?
- Does Sry, derived from Sox3, still need to maintain a Sox3 expression level until X-inactivation?
- Do Sry and Sox3 differences in the nervous system act in a sex determination mechanism during development of the nervous system?
- Do effects of Sry on the sympathetic nervous system predispose males to an increased response level?
- What is the developmental expression of Sry and how are these expression profiles conserved across mammalian evolution?
- Is Sry responsible for sex differences in human disease? Sex differences have been described for many diseases in addition to hypertension, including myocardial infarction (93), stroke (3), and many immune disorders (98).
- Is Sry involved in autism risks since there are a male excess and Y chromosome haplotype associations (84). Could Sry expression in the nervous system be responsible?
- Could Sry expression affect chronic kidney disease? Males have a higher incidence and progress to end-stage renal disease faster than women (49, 62). Diabetic males are more likely than females to show renal damage (75).
- Is Sry involved in other conditions that have a major sex differences? For example, males have higher mortality following hip fracture (67), shorter time to secondary progressive multiple sclerosis (44), faster disease progression following HIV seroconversion (39), higher incidence of small intestinal cancer (71), worse prognosis in idiopathic pulmonary fibrosis (10), and increased risk of abdominal aortic aneurysm (41).
- Will Sry expression be shown to be responsible for all sex differences? No, but the transcriptional effects of Sry expression in adult males should at least be considered as a potential option. While some of these differences may be a reflection of hormonal status or environment, others likely represent genetic or epigenetic differences.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**REFERENCES**


apoA1, more than testis determination?

R569


Perspectives

Sry, MORE THAN TESTIS DETERMINATION?


