CMLS, Cell. Mol. Life Sci. 55 (1999) 839–856
1420-682X/99/070839-18 $ 1.50 + 0.20/0
© Birkhäuser Verlag, Basel, 1999

CMLS Cellular and Molecular Life Sciences

Sry and *Sox9*: mammalian testis-determining genes

P. Koopman

Centre for Molecular and Cellular Biology, The University of Queensland, Brisbane, QLD 4072 (Australia), Fax + 61 7 3365 4388, e-mail: p.koopman@cmcb.uq.edu.au

Abstract. *Sry* is the Y-chromosomal gene that acts as a trigger for male development in mammalian embryos. This gene encodes a high mobility group (HMG) box transcription factor that is known to bind to specific target sequences in DNA and to cause a bend in the chromatin. DNA bending appears to be part of the mechanism by which *Sry* influences transcription of genes downstream in a cascade of gene regulation leading to maleness, but the direct targets of *Sry* remain to be positively identified. One gene known to be downstream from *Sry* in this cascade is *Sox9*, which encodes a transcription factor related to *Sry* by the HMG box. Like *Sry*, mutations in *Sox9* disrupt male development, but unlike *Sry*, the role of *Sox9* is not limited to mammals. This review focuses on what is known about the two genes and their likely modes of action, and draws together recent data relating to how they might interconnect with the network of gene activity implicated in testis determination in mammals.

Key words. *Sry*; *Sox9*; sex determination; *Sox* genes; testis; mammalian; review.

Discovery of *Sry*, the Y-linked testis determinant

It has been known for some decades that in mammals, maleness is determined by the Y chromosome. Cytogenetic analyses in mice and humans showed conclusively that the Y chromosome carries a genetically dominant locus that normally induces male development in XY individuals [1–3]. This locus was given the acronym *TDF* (*testis-determining factor*) in humans and *Tdy* (*testis-determining gene on the Y*) in mice, and is now known to harbour the gene *Sry* (denoted *SRY* in humans).

The molecular strategy used to narrow down the region of search and eventually isolate the gene *SRY* is now regarded as a classic success story of positional cloning. Most positional-cloning projects involve identification of a candidate gene by analysis of translocations and deletions that result in an abnormal phenotype. In the case of sex determination, this was most readily approached by studying sex reversal in humans. The majority of human XX males possess Y-derived DNA sequences, transferred to the paternal X chromosome by aberrant X-Y interchange during meiosis [4, 5]. When DNA from four such XX males was analyzed, all were positive for Y-specific markers located in the 35 kb immediately adjacent to the pseudoautosomal boundary [6]. This 35-kb region was searched for conserved Y-linked sequences, an open reading frame found, and the corresponding gene dubbed *SRY* (*sex-determining region Y* gene). A homologous gene was found on the mouse Y [7].

This gene had all the properties expected of *TDF*, as elaborated in the following sections. Importantly, mutations in *SRY* were identified in XY females, showing that *SRY* function is normally required for testis development [8, 9]. The role of *Sry* in testis development was confirmed in transgenic mouse experiments, in which XX mice bearing *Sry* were able to develop as males [10]. These experiments demonstrated that only one gene from the Y chromosome is necessary and sufficient to initiate the cascade of male development, and that *Sry* encodes the testis-determining factor.

Structure and function of SRY

The role of the HMG box: DNA binding and bending

Sequence analysis of *Sry* revealed a region encoding a 79-amino acid motif that has come to be known as an HMG box, due to its presence in some of the high-mo-

bility-group class of nonhistone proteins that associate with DNA [11]. This observation placed SRY among a set of sequence-specific DNA-binding transcription factors that include TCF1 and LEF1 [12–14]. Human SRY protein was shown to bind specifically to the sequences AACAAAG and, preferentially, AACAAT, the concensus binding sites of TCF1 and LEF1, respectively [15–18]. Mutations associated with XY sex reversal in humans were found to impair the binding of SRY protein to these target sites in electrophoretic mobility shift assays [16], indicating that the ability to bind to DNA is an integral part of the biochemical mechanism by which SRY directs male development. These observations sat well with expectations that the testis-determining factor was a cell-autonomous regulatory molecule at the top of a cascade of gene regulation leading to testis formation.

Further study revealed that SRY protein from humans and mice is able to induce bending of DNA by angles of 60–85° [19–21]. Similar properties were reported for LEF1 [20], leading to the suggestion that these proteins may act as 'architectural' transcription factors, which are thought to act by influencing chromatin structure in the regulatory regions of target genes, allowing the assembly of regulatory complexes [22, 23]. Elegant in vitro studies showed that the activity of LEF1 in regulating the *TCR*α enhancer depended critically on the relative placement of its binding site and those of two cofactors, Ets-1 and PEBP2α, within the enhancer [24], as would be expected if the above model is correct. *Sry* was able to partially replace *LEF1* in stimulating enhancer function in transfection assays. Mutant SRY proteins produced by some human XY females have been found to be defective not in DNA binding, but in their ability to bend DNA [25], in support of an architectural model of transcriptional regulation by SRY.

What does the remainder of the SRY protein tell us about its likely mode of action in sex determination? In short, very little. The non-HMG box sequence of SRY is remarkably poorly conserved between species (fig. 1). This is partly explicable by the location of *Sry* on the Y chromosome, conducive to a greater degree of sequence drift than that seen in autosomal or X-linked genes. Even so, the frequency of nonconservative substitutions in SRY is far greater than expected, to the extent that some workers have postulated positive selection for sequence change in *Sry* during evolution [26, 27]. Not only the primary structure but also the general topology of SRY can vary dramatically between species, in terms of the sizes of the regions N- and C-terminal to the HMG domain (fig. 1). Further, mutations in human *SRY* that result in XY sex reversal are almost exclusively found in the HMG box (fig. 2). Taken together, these observations point to a reliance of SRY largely if not solely on the HMG box for function, and an architectural mode of action seems most likely.

Transcriptional activator or repressor?

While the conventional view is that SRY is likely to set in train a pathway of gene regulation leading to testis determination and differentiation by activating one or more downstream genes, an alternative suggestion is that SRY may act as a transcriptional repressor. In this latter model, SRY may act to repress genes that activate the female pathway of development, or to repress a

Figure 1. Sequence and topological comparisons of SRY proteins from various mammalian species. Linear schematic diagrams of predicted proteins are shown, with the HMG box in black. Numbers show amino acid identity to human SRY in the N-terminal, HMG box and C-terminal regions of the proteins, respectively. Sequences were sourced from GenBank, accession numbers L08063 (human), X86380 (*Pan troglodytes*, chimp), AB 004572 (*Equus caballus*, horse), U70655 (*Mus musculus musculus*, mouse), and S46279 (*Sminthopsis macroura*, stripe-faced dunnart). Identities were calculated using the University of Wisconson Genetics Computer Group program GAP, accessed through the Australian National Genome Information Service. The mouse protein contains a large, glutamine-rich repetitive region shown as a cross-hatched box. Mouse SRY in the subspecies *M. m. domesticus* has a premature stop codon that truncates this domain at the position indicated by an asterisk.

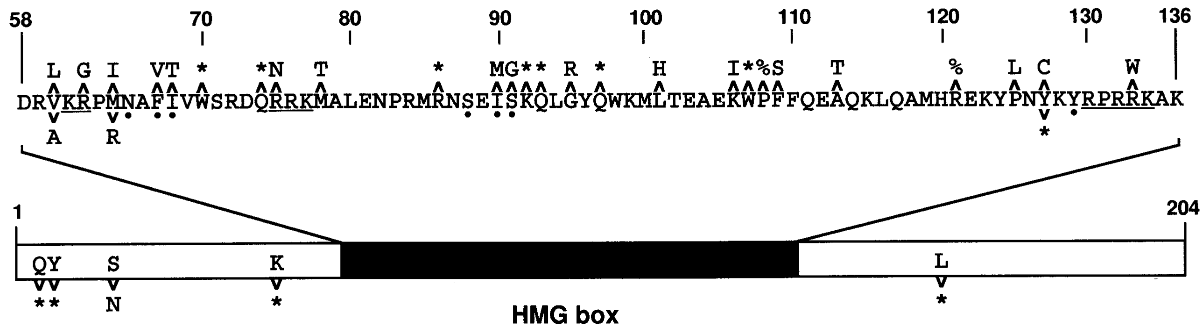

Figure 2. Sex-reversing mutations in human SRY leading to gonadal dysgenesis or hermaphroditism. A linear schematic diagram of the human SRY protein is shown, with the HMG box in black. The 79-amino acid HMG box sequence is shown above in single letter code. Missense mutations are denoted by the substituted amino acid above or below the normal sequence, nonsense mutations by '*' and frameshifts by '%'. Note that the vast majority of these mutations affect the HMG box. The effects of many of these on the structure and function of the HMG box have been described [16, 25, 69, 166]. Amino acids involved in DNA contact are indicated with a dot [166]. The bipartite and basic cluster nuclear localization signals [33, 34] are underlined. Mutation data are from literature current to December 1998 [8, 9, 38, 69, 71, 167–184].

repressor of the male pathway. The repressor model is supported by genetic studies of XX maleness. The frequency of XX males in the human population is about 1 in 20,000 [28], and it is estimated that 10% of these cases are *SRY*-negative [29, 30], implying that the frequency of SRY-negative XX maleness is around 1 in 200,000. This frequency is generally agreed to be too high to be explained by sporadic gain-of-function mutations in male-determining genes downstream from *SRY*, and is perhaps more likely to result from loss-of-function of a repressor of maleness downstream from *SRY* [31]. As yet no transcriptional repressor has been identified in the male sex-determining pathway, nor is there any a priori reason why such a repressor needs to be a direct target of SRY. However, the postulated repressive mode of action of SRY is compatible with the implication that it acts by virtue of the HMG box alone; SRY protein could compete with or otherwise block the binding of another regulatory factor, or may impede rather than facilitate the assembly of a transcriptional complex through its bending properties. In vitro evidence that SRY can act as a conventional repressor of transcription has also been presented [32].

Nuclear localization and phosphorylation

Assuming for the moment that SRY exerts its effects through the HMG domain alone, can other essential features of a transcription factor be ascribed to the HMG box? One of these features is the ability of the protein to be transported to the nucleus after assembly in the cell cytoplasm. Recently, two independent nuclear localization signals have been identified at opposite ends of the HMG box of SRY [33, 34]. Some human XY females carry mutations in these nuclear localization signals (fig. 2). In one case (R62G), transfection of a mutant protein expression construct into COS-7 cells revealed no differences in nuclear localization compared with wild-type constructs [34]. It remains to be seen whether other mutations do impede nuclear transport, implicating a mode of sex reversal additional to reduced DNA binding or bending by the HMG box.

Phosphorylation is one of the major mechanisms by which the activity of transcription factors can be modulated in the cell. Recently, the cyclic AMP-dependent protein kinase (PKA) has been found to phosphorylate human SRY protein on serine residues located in the N-terminal part of the protein [32]. This phosphorylation event was shown to positively regulate SRY DNA-binding activity, providing evidence for posttranslational modulation of SRY activity during sex determination. Curiously, the PKA phosphorylation site (RRSSS) is conserved among primates but not other mammalian orders.

Interaction of SRY with other proteins

A further essential requirement is that SRY possess a mechanism by which it can specifically act on its target genes. SRY is a member of a large family of transcription factors, known as SOX proteins, related by similar HMG box sequences. One of these, SOX9, will be discussed in some detail below. All SOX proteins characterized to date can bind to similar target sequences,

and functional specificity is presumably achieved through interaction with distinct cofactors in different regulatory contexts [35, 36]. Two interacting factors have so far been described for SRY. One of these is a nuclear factor, SIP1, containing PDZ protein interaction domains [37]. SIP1 was found to interact not with the HMG box, but instead with the C-terminal seven amino acids of human SRY. These amino acids are conserved in chimpanzee and gorilla SRY, but not in gibbon, orangutan or any of the other mammalian SRY proteins characterized to date. A base substitution generating a premature stop codon C-terminal to the HMG box in human SRY and resulting in XY gonadal dysgenesis has been described [38]. Such a mutation would affect the putative SIP1 interaction motif, providing a possible explanation for the phenotype, but may also affect other motifs or alter protein stability. In a separate study, Harley and colleagues found that the HMG box of SRY is a calmodulin binding domain [39]. Calmodulin interacts with a nuclear localization signal, and may therefore modulate nuclear import of SRY ([39] and V. Harley, personal communication).

Despite the overwhelming evidence regarding the importance of the HMG box for SRY function, a second, highly conspicuous domain is found in mouse SRY (fig. 1). This domain in the *Mus musculus molossinus*-derived Y chromosomes, found in common laboratory strains such as 129, occupies over half the SRY protein (223 of 395 amino acids) at its C-terminus, is encoded mainly by a CAG trinucleotide repeat and is rich in glutamine and histidine residues [40]. This sequence is arranged as 19 blocks of 2–13 glutamine residues interspersed by a conserved highly polar spacer of sequence FHDHH or similar. This domain is able to function as a transcriptional activator in a GAL4 assay in cultured cells [41]. Balanced against this observation is the absence of this domain in all other genera, and the finding that no substitute trans-activation domain exists in human SRY [41], suggesting that the glutamine-rich region does not play an activation role, nor indeed any role, in vivo. Even within the species *M. musculus*, this domain is variable: a stop codon truncates the glutamine-rich repeat region less than halfway through in *domesticus* subspecies [42] (fig. 1). This truncated domain is unable to activate transcription in vitro [43]. We have tested directly whether the glutamine-rich repeat domain of mouse SRY is functionally relevant, using a transgenic mouse assay (J. Bowles and P. Koopman, unpublished analysis). Constructs lacking this domain were unable to induce sex reversal, suggesting that the glutamine-rich repeat domain is essential for mouse SRY function, and that mouse SRY protein differs in its biochemical mode of action from SRY in other species. In support of this conclusion, a protein that specifically interacts with the glutamine-rich repeat region of mouse SRY has recently been reported, but details relating to the nature of this protein and its mode of action have not been published [44].

In summary, while it appears that SRY protein acts via its HMG domain to influence the transcription of other genes, basic information pertaining to what this influence is and how it is brought about is still lacking. Clearly, target genes need to be identified as a priority in further clarifying the mode of action of SRY as a transcription factor.

Expression of *Sry*

Temporal and spatial profile of *Sry* transcription

In order to bring about male sex determination, *Sry* needs to be active in the gonadal primordia, known as the genital ridges, of the XY embryo. Shortly after the cloning of *Sry*, it was established that this gene is indeed expressed in the somatic cells of the genital ridges in mouse embryos [45]. This expression begins soon after the genital ridges first arise, about 10.5 days post coitum (dpc), reaches a peak around 11.5 dpc and is maintained only until the first morphological signs of testis differentiation become apparent at about 12.5 dpc [45–47]. Expression in mice was found to be specific to the genital ridges [48], although low-level expression in mouse, bovine and human blastocyst-stage embryos has been noted [49–51]. This expression profile is compatible with SRY acting specifically in the genital ridges to trigger a pathway of gene expression leading to testis development, with no continuing requirement for SRY expression in the maintenance of that pathway.

Once again, mice may not be truly representative of all mammals in this regard. A wide variety of expression profiles have been noted among different mammalian species. In humans, marsupials and sheep, *Sry* transcription appears to be much less tissue- and stage-specific than in mice. For example, the timing of *Sry* transcription in sheep and Tammar wallabies encompasses that of testis differentiation by a broad window of several days either side [52, 53]. In humans and wallabies, expression in several fetal and adult tissues has been noted [52, 54]. These observations suggest either that *Sry* has roles other than in sex determination in these species, or that expression outside of the genital ridge or beyond the narrow time window of sex determination has no functional relevance. If the former explanation is true, the effects of *Sry* in nongonadal tissues must be subtle, as mutation and indeed absence of *Sry* appears only to affect the testes and processes depending on the hormonal output of the testes. It may well be that the relatively extensive expression of *Sry* in nonmurine species is redundant, and that mice have retained only the minimal expression profile required for male sex determination.

Consistent with this view is another curious quirk of *Sry* expression in mice. In the course of attempts to isolate a complementary DNA (cDNA) for *Sry*, libraries were screened from the most abundant source of *Sry* expression, the adult testis. cDNA clones were repeatedly generated in which sequences normally located 3′ to the HMG box were found in a 5′ position, abutting sequences upstream from the HMG box. These results were confirmed by RNase protection and rapid amplification of cDNA ends-polymerase chain reaction (RACE-PCR). This apparent conundrum was solved by the demonstration that a circular transcript is produced in adult testes [55]. Circularization is thought to result from the unique structure of the *Sry* locus: mouse *Sry* is embedded in a large inverted repeat [40]. The adult testis transcript is believed to initiate within one arm of this repeat [56], encompass the open reading frame and terminate in the other arm of repeated sequence. This scenario would generate a stem-loop transcript, and a splice involving donor and acceptor sequences present in the loop could generate the final circle [55, 57]. Why this occurs in mice is not known. The circular transcript is not translated and therefore appears to be redundant, in contrast to the functional transcript produced in the genital ridges, and may be used as a means of suppressing *Sry* function in sites of gene expression other than the fetal gonads. However, evidence from transgenic studies in my laboratory indicates that loss of tissue specificity of *Sry* expression is not detrimental in mice (J. Bowles and P. Koopman, unpublished analysis).

The primordial gonad is composed of a number of distinct lineages, each of which has the potential to differentiate into testicular or ovarian counterparts, depending on signals received. The primordial germ cells can either mitotically arrest and subsequently develop into prospermatogonia, or enter meiosis and become oogonia [58]. This decision is a result rather than an effector of sex determination, as embryos lacking germ cells are able to undergo somatically normal sex determination [59, 60]. Clearly germ cells cannot be the site of *Sry*'s action, and indeed mouse mutant embryos lacking germ cells retain *Sry* expression [45]. Somatic cell lineages in the developing gonads include supporting cell precursors that can develop into Sertoli cells in males or follicle cells in females, steroidogenic precursor cells that go on to become either Leydig cells or theca cells, and mesenchymal cells that can either contribute to the peritubular myoid cells and vasculature of the testis, or organize into ovarian connective tissue and vasculature. Genetic studies involving XX↔XY chimeric mice have shown that of these cell types, *TDF*/*Tdy* expression is required in a cell-autonomous fashion only in the supporting cell lineage [61, 62]. These studies were supported by direct observation of *Sry* expression in Sertoli cell precursors [63]. These results imply that the role of SRY is to act as a switch that influences the differentiation of Sertoli cells, and that factors produced by differentiating Sertoli cells influence the differentiation and organization of the remaining cell lineages.

Importance of expression levels for *SRY* function

Many lines of evidence combine to indicate that expression levels of *Sry* are important for sex determination. In humans, different degrees of masculinization can be seen in XX siblings inheriting the same Y chromosome-derived fragment after aberrent meiotic X-Y interchange in their father's germ line [64]. This variation must be due either to different degrees of mosaicism for cells inactivating the X chromosome bearing *SRY* in the Sertoli cell lineage, or differences in *SRY* expression levels due to differential spreading of X chromosome inactivation, or both. In mice, XY sex reversal is associated with two types of mutation that appear to exert their effects through suppressing *Sry* expression levels. Capel and colleagues noted that deletion of repeat sequences at some distance proximal to *Sry* on the mouse Y short arm result in reduced expression and sex reversal [65]. More recently, the sex reversal seen when crossing an *M. m. musculus* strain *Poschiavinus* Y chromosome (Y^{POS}) onto an *M. m. domesticus* background (in particular C57BL6) [66] has been ascribed to reduced *Sry* expression levels from Y^{POS}. In an elegant study, Nagamine and colleagues showed by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) that levels of *Sry* expression from different mouse Y chromosomes correlated with the degree to which they cause sex reversal on a *domesticus* background (C. Nagamine et al., unpublished data). It appears that a critical threshold must be achieved by a certain stage in genital ridge development for the supporting cell population to be pushed towards Sertoli cell differentiation.

Regulation of *Sry* transcription

While expression levels of *Sry* are clearly important, it is not yet known how *Sry* is regulated. Experimental attempts to define the regulatory regions of *Sry* have been hampered by poor conservation of upstream sequences, low expression levels, lack of suitable cell lines to use in transfection studies and unsuitability of β-galactosidase as a reporter molecule for *Sry* expression in transgenic mice. Comparison of upstream sequences from closely related species such as primates has identified some conserved elements that may be relevant to *Sry* regulation [67, 68]. Mutation searches in *SRY* upstream sequences in cases of XY sex reversal have typically found no sequence changes [69–72]. Poulat

and coworkers [72] found one point mutation at position −75 in an XY female, but the significance of this mutation is unclear, as the father's DNA could not be analyzed. Certainly, deletion of 33–60 kb of upstream sequence, starting 1.8 kb 5′ to *SRY*, causes XY sex reversal [73]. Downstream sequences may also be implicated in *SRY* regulation in humans [71, 74]. Whatever the eventual outcome of *Sry* regulatory studies, important cis sequences and their trans-acting regulatory factors identified in one species such as mice may or may not be broadly relevant, given the differences in *Sry* structure and expression between species. Human *SRY* is unable to cause male sex determination in transgenic mice [10], and it is not yet known whether this is due to structural or regulatory incompatibilities between the human gene or protein and the host mouse cells. Clearly, however, investigations relating to how the *Sry* gene is regulated pose a formidable challenge.

Evolution of *Sry*

What is the origin of *Sry* and how did it come to control a decision of such fundamental importance for the individual and for the species? Obviously it is possible only to speculate on these questions, based on available evidence. First, *Sry* is found only in mammals. Extensive searches among other vertebrate classes for genuine orthologues of *Sry* (that is, genes containing an HMG box that are present in one sex but not the other) have yielded only *Sox* genes that are not sex-specific [75, 76]. Many eutherian and a few marsupial and monotreme species have been studied, and *Sry* has been found in all of these bar three, namely two vole species, *Ellobius lutescens* and *E. tancrei* [77], and the spiny rat *Tokudaia osimensis* [78]. Assuming that these species have diverged on their own evolutionary tangents, it seems that the origin of *Sry* is inextricably linked to the genesis of the class Mammalia.

The X and Y chromosomes are thought to have arisen as a pair of autosomes that began to diverge when the Y took on a male-determining function. *Sry* may thus have begun its life as a *Sox* gene on the ancestral autosome. The X chromosome of humans, mice and marsupials carries a gene, *Sox3*, which may be the gene from which *Sry* evolved [79–81]. *Sox3* is primarily expressed in the developing central nervous system, although transcripts have been reported in the genital ridges of both sexes in mice and chickens [81–84]. It is tempting to speculate that *Sox3* may once have been, or may still be, involved in female sex determination, and that competition between SRY and SOX3 proteins was or is the basis of a male-female dichotomy. There is currently no evidence for a role for *Sox3* in determination of either sex [79, 85]. However, it is not necessary for *Sox3* to have such a role in order to have been the ancestor of *Sry*, as the latter may have taken on an entirely novel function. Further, given the rate of evolution of *Sry*, it is impossible to say with certainty which *Sox* gene *Sry* most resembles. It is not yet known whether other *Sox* genes reside on the X chromosome and can be considered as possible ancestors of *Sry*.

Relationship of *Sry* to other genes implicated in sex determination

A number of genes have been identified that have a critical role in the male or female sex-determining pathway, and may therefore interact in some way with *Sry*. These are described briefly below, and three—*DAX1*, *SF1* and *WT1*—are discussed in detail elsewhere in this volume. A schematic diagram of the cellular and molecular interactions during sex determination and gonadal development is shown in figure 3.

Amh

Anti-Müllerian hormone (AMH, otherwise known as Müllerian-inhibiting substance or MIS) causes regression of the Müllerian (female) duct system. Because it is the first identifiable Sertoli cell product and appears soon after the onset of *Sry* expression, it was for some time suspected that the *Amh* gene is directly regulated by SRY. However, it now appears that at least intermediaries such as SOX9 and SF1 are involved (see below). In any case, it is known that AMH is not an integral link in the male-determination pathway, as mice lacking AMH show normal testis development [86].

SF1

The orphan nuclear receptor steroidogenic factor 1 (SF1), otherwise known as Ad4BP, is a key component of the pathway of gonadal and adrenal development, and is known to regulate male steroid biosynthesis. *Sf1* transcripts are present in the gonads of both sexes in mice between 9 and 12 dpc, whereafter the gene is downregulated in ovaries [87, 88]. Mice homozygous for a null allele in *Sf1* show normal early development of the genital ridges, but a complete block in subsequent gonadal development in both sexes, among other defects [89]. The *Amh* promoter contains an SF1 binding site which is critical for expression of the gene in Sertoli cells in vitro and in transgenic *Amh*-reporter mice [90, 91]. These data suggest that SF1 has at least three important roles in sexual development, one early in the establishment of the gonad prior to the expression of *Sry*, another later in male differentiation, regu-

lating *Amh*, and another in regulating steroid synthesis in Leydig and theca cells.

WT1

Targeted disruption of the Wilms's tumour-associated zinc-finger gene *WT1* also results in a blockage of gonad development in mice [92]. As with *Sf1* deficiency, the initial stages of genital ridge formation are unaffected, suggesting that *SF1* and *WT1* play important roles in the maturation of the genital ridges, rather than sex determination per se, perhaps by establishing an environment in which *Sry* can act to commit cells to the male fate. In humans, dominant-negative *WT1* mutations are associated with XY pseudo-hermaphroditism in Denys-Drash syndrome [93]. It has been suggested that WT1 may act as a regulator of *Sry* expression [94], and potential WT1 binding sites are present upstream of both mouse and human *Sry*. However, direct evidence for this regulation is lacking.

LIM1

LIM1 is a homeobox gene that regulates production of an organizing molecule in many experimentally studied species. Targeted disruption of *Lim1* in mice resulted in complete absence of head structures and early lethality, but surviving fetusus lack kidneys and gonads [95]. It is widely assumed that *LIM1*, like *WT1* and *SF1*, is involved in maturation of the genital ridges, but its precise role in gonadal induction has not been analyzed in detail, due in part to the lethality of *Lim1* knockout embryos.

DAX1

Another orphan nuclear receptor gene, *DAX1* maps to a region of the human X chromosome which, when duplicated, causes male-to-female sex reversal [96–98], and it has been suggested that *Dax1* is an ovarian-determining gene [96]. Deletions involving *DAX1* do not disrupt testis differentiation, and *Dax1* expression in

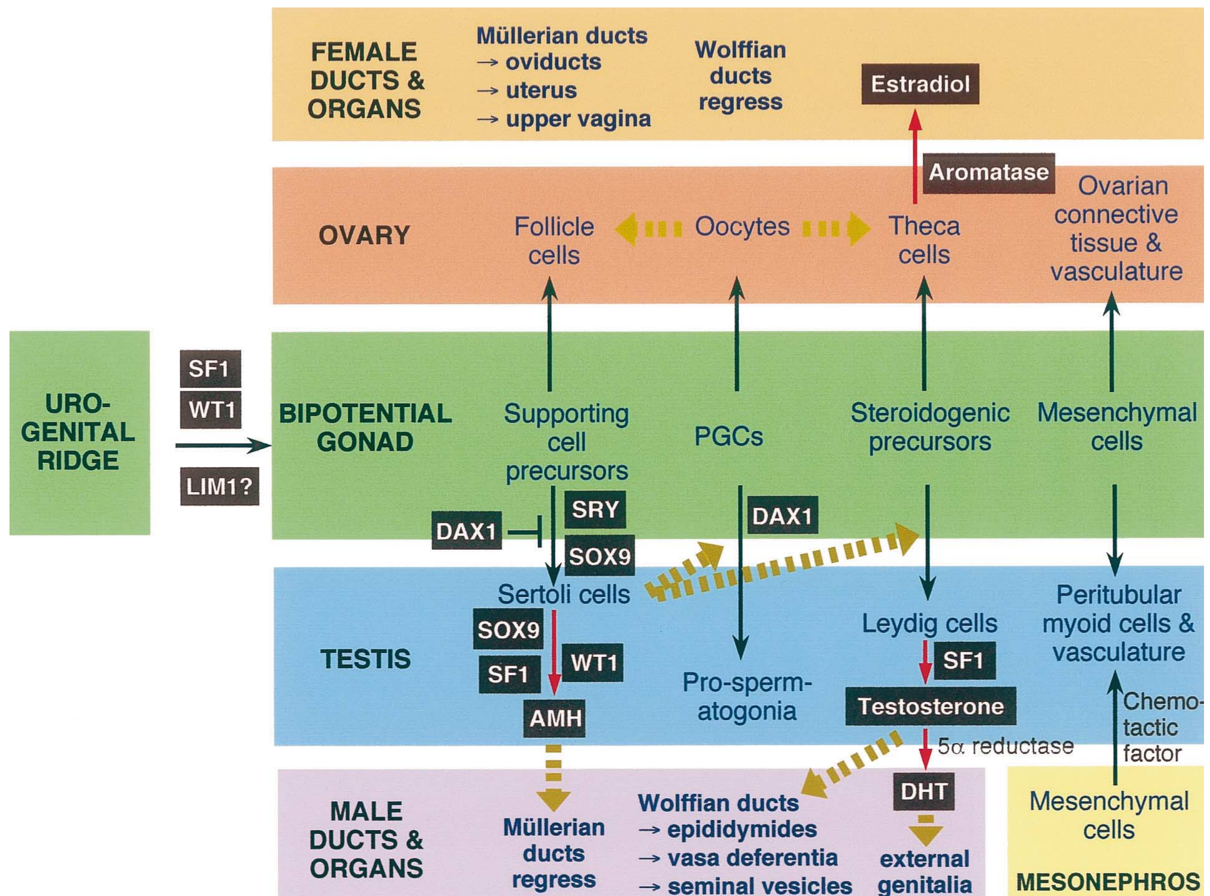

Figure 3. Cellular and molecular interactions during gonadal induction. Pathways of cellular differentiation and/or migration are indicated by black arrows, biosynthetic pathways by red arrows, and hormonal or unknown signalling pathways by large dotted arrows. Effector genes or gene products are shown in brown boxes. DHT, dihydrotestosterone.

mice is downregulated with testis differentiation, but persists in the developing ovary [99]. Transgenic mice overexpressing *Dax1* have been shown to undergo male-to-female sex reversal, suggesting that *Dax1* and *Sry* act antagonistically [100]. However, targeted inactivation of *Dax1* in mice does not affect ovarian development, instead blocking spermatogenesis in males [101]. It is concluded that *Dax1* is a spermatogenesis and antimaleness gene rather than an ovarian-determining gene [100, 102].

Aromatase

In vitro binding of SRY to the *aromatase* promoter has also been reported [103]. Aromatase is a member of the cytochrome P-450 family and is responsible for the conversion of testosterone to estradiol. It is considered essential for male sex determination that the gene encoding aromatase be tightly repressed during testis development, and Haqq et al. have suggested that SRY is directly responsible for this repression. This is unlikely to be the case in vivo; *SRY* expression occurs in Sertoli cells, yet aromatase is a product of the steroidogenic lineage (fig. 3).

Fra1

The *Fos*-related antigen gene *Fra1* has been implicated as a target of SRY, since transfection of a plasmid expressing human SRY protein significantly enhanced transcription of a *Fra1* reporter construct [104]. However, *Fra1* is not expressed at any stage at or around the critical period of sex determination when *Sry* transcripts are present in mice, excluding a role for *Fra1* in sex determination and differentiation [47].

Sox9

As mentioned earlier in this review, *Sry* is just one of a family of genes related by the HMG box. These genes have come to be known as *Sox* (*SRY-related HMG box*) genes (for review, see [105, 106]). A rule of thumb for defining *Sox* genes, as distinct from other HMG box genes, is that they are more than 50% identical to SRY at the amino acid level in the HMG box region [105, 106]. However, this cannot be regarded as strictly accurate, as several exceptions to this rule exist [107] (P. Koopman, unpublished analysis). *Sox* genes have been found in a wide variety of species representing insects, amphibians, ascidians, reptiles, birds, fishes and mammals. They are best characterized in mice, with some 20 *Sox* genes identified in whole or part in that species [7, 107–117]. Expression studies [35, 45, 81, 113, 115, 117–126], knockout mouse experiments [127, 128] and the identification of mutations in a number of human diseases [8, 9, 129–131] indicate that *Sox* genes are important for the differentiation and/or function of a number of cell types during embryonic development.

One *Sox* gene of special relevance for sex determination is *Sox9*. Expression of *Sox9* was first noted at sites of chondrogenesis in mouse embryos [132]. In humans, heterozygous defects in *SOX9* have been associated with the bone dysmorphology syndrome campomelic dysplasia (CD) [129, 130]. Curiously, a large proportion of CD patients show XY sex reversal [133, 134], demonstrating an important role for *Sox9* not only in skeletal development but also in sex determination. *Sox9* is one of only a few genes apart from *Sry* for which mutations have been shown to interfere with male sex determination.

Structure and function of SOX9

The HMG box: DNA binding and nuclear localization

The structure of SOX9 is that of a typical transcription factor with discrete DNA binding and transcriptional trans-activation domains. The HMG box region of SOX9 has been shown to bind to the sequences AACAAT and AACAAAG [35, 135], typical of SRY and other SOX proteins. In addition, SOX9 has been found to bind to the variant sequences ATGAAT and CACAAT, found in the chondrocyte-specific enhancer in the first intron of the human type II collagen gene *COL2A1* [135, 136]. Mutation of these binding motifs abolishes chondrocyte-specific expression of a *COL2A1* reporter in transgenic mice [135], demonstrating not only the importance of SOX9 binding for *COL2A1* expression, but also that the canonical SOX binding site (WWCAAWG, where W = A or T) defined by binding site selection experiments in vitro is not necessarily used in vivo.

The importance of DNA binding for SOX9 function is reflected by the presence of mutations affecting the SOX9 HMG box in sex-reversed (XY female) CD patients [129, 130, 137–139] (fig. 4). Impaired DNA binding affinity has been demonstrated in a number of these cases [138]. It is likely that SOX9 binding results in chromatin bending, but bending brought about by normal SOX9, let alone impaired bending resulting from sex-reversing mutations in the HMG box, has not been investigated.

The HMG box of SOX9, like that of SRY, contains two independent nuclear localization signals [34] (fig. 4). In mice, SOX9 protein appears predominantly cytoplasmic in the genital ridges of both sexes prior to 11.5 dpc, but moves to the nucleus at later stages of testis development [120], and it has been suggested that a factor or factors that interact with the nuclear localization signal(s) may mediate this change in subcellular localiza-

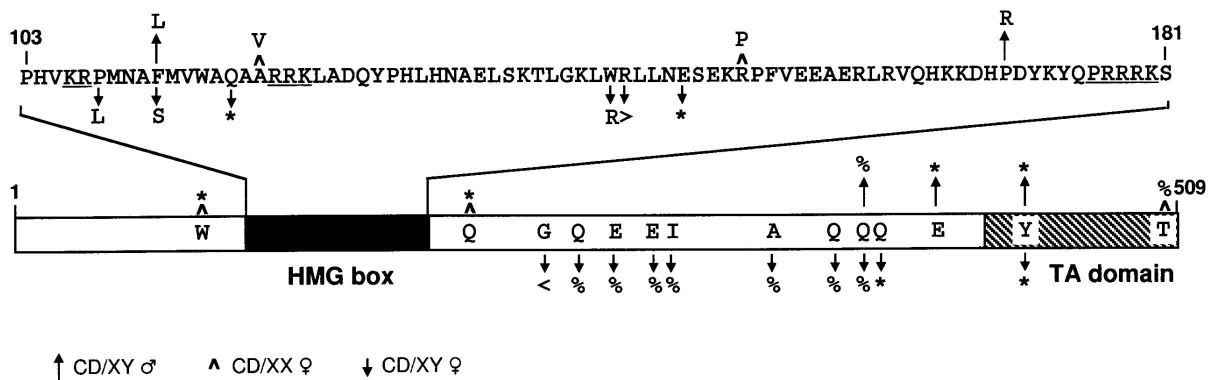

Figure 4. Mutations in human *SOX9* associated with campomelic dysplasia and XY sex reversal. Linear schematic diagram of the 509-amino acid human SOX9 protein. The HMG box and trans-activation (TA) domain are indicated, with the 79-amino acid HMG box sequence shown above in single letter code. Missense mutations are denoted by the substituted amino acid, whereas nonsense mutations are denoted by '*' and frameshifts by '%'. Mutations affecting splice donor (<) and acceptor (>) sequences have also been reported. Mutations designated by an upward arrow do not affect sex determination (i.e. they are present in XY males). Those designated by a downward arrow result in complete or partial XY sex reversal (XY females). The effect on male sex determination of the mutations designated ^ is not known (XX females). The bipartite and basic cluster nuclear localization signals [34] are underlined. Mutation data are from literature current to December 1998 [129, 130, 137–139, 185, 186].

tion [34]. No direct support for this theory has emerged, and mutations specifically affecting nuclear localization sequences of SOX9 have not yet been detected among campomelic dysplasia patients.

Transcriptional trans-activation

Sex-reversing mutations in *Sox9* are found throughout the gene, with no correlation between the type of mutation and the phenotype [138] (fig. 4). Clearly, functional domains outside of the HMG box are implicated by these mutations. In vitro GAL4 activation assays have identified the C-terminal 108 amino acids in human SOX9 as a trans-activation domain [140]. In mice, the C-terminal 83 amino acids are capable of trans-activation [35]. The difference is likely due to the serendipitous choice of gene fragments assayed in the respective laboratories rather than to any genuine differences between the mouse and human proteins, and it may be that the real size of the trans-activation domain is smaller still. In support of this possibility, the C-terminal 43 amino acids of the SOX9 protein are particularly well conserved between the human, mouse, chicken and rainbow trout sequences (fig. 5). Whatever the actual size of the trans-activation domain, all of the CD mutations C-terminal to the HMG box are termination or frameshift mutations that would debilitate the trans-activation ability of the protein. It would appear then that SOX9 is able to function as a conventional transcription factor, but whether the protein has additional or alternative architectural roles remains to be seen.

Multiple roles of SOX9

Unlike the situation in SRY, SOX9 shows a very high degree of sequence conservation throughout the protein between species. This conservation is typical of *Sox* genes other than *Sry*, and is particularly high for the HMG box and trans-activation domains (fig. 5), as might be expected. Sequence comparisons also reveal several other very highly conserved regions that may serve as interfaces for interaction with other proteins. One might predict that SOX9 would interact with a variety of cofactors in a combinatorial fashion to achieve its different roles, presumably involving many different regulatory targets, during development [35]. For example, SOX9 is known to directly activate *COL2A1* and the gene encoding the matrix component aggrecan during chondrogenesis [135, 136, 141]. Cooperation with SOX5 and/or SOX6 synergizes the activation of *COL2A1* [142], and other, as yet unidentified cofactors may be involved in *COL2A1* activation. Cofactors enhancing the effect of SOX9 on *aggrecan* transcription are not yet known. In sex determination, SOX9 would be expected to activate an entirely different gene or set of genes; certainly, *COL2A1* is not expressed in developing testes [35]. These data provide further support that the sex-determining cascade is better thought of as a combinatorial network rather than a linear pathway.

Curiously, only some 75% of XY infants showing the skeletal abnormalities diagnostic for CD are sex-reversed [143]. In other words, some *SOX9* mutations result in CD with XY sex reversal and others in CD

without XY sex reversal, whereas mutations seen in XX patients are uninformative with respect to their effects on male sex determination (fig. 4). This may mean that the molecular cascade of chondrogenesis is more sensitive than that of sex determination to perturbations in SOX9 function. Alternatively, the majority of CD fetuses may develop ovotestes, which resolve to either testes or ovaries after birth, as occurs in mice. No cases

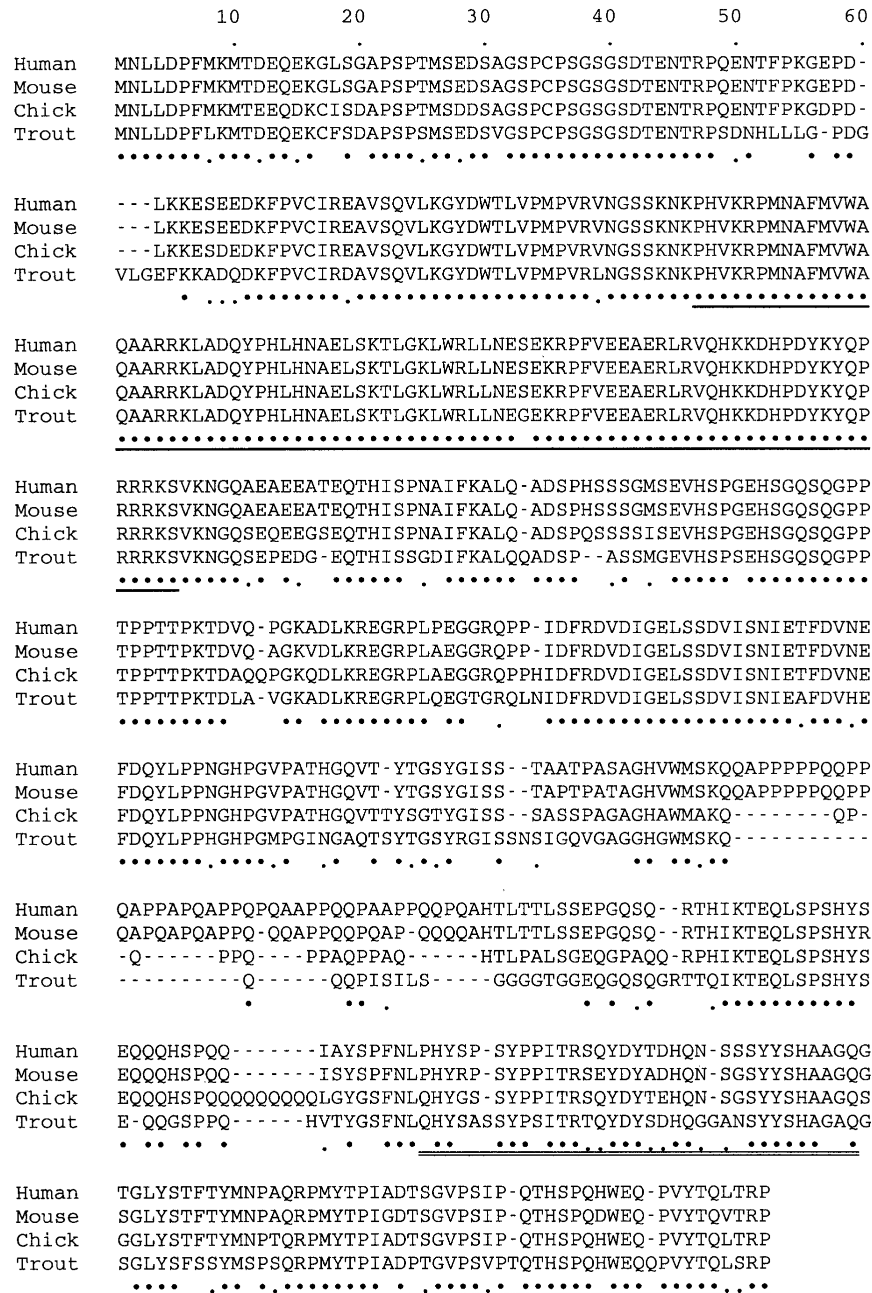

Figure 5. Comparison of SOX9 proteins from human, mouse, chicken and trout. Amino acids identical in all four sequences are indicated with a bold dot, and those subject to conservative change in some sequences with a smaller dot. The HMG box is underlined, and the N-terminal 83-amino acid region implicated in transcriptional trans-activation in the mouse is double-underlined. The pattern of conservation through the SOX9 protein in these diverse vertebrates suggests that regions other than the HMG box and trans-activation domains may be of functional significance. Data from [36, 129, 132, 187].

of *SOX9* mutations resulting in sex reversal without CD have been found [138, 144], and the nature and distribution of the sex-reversing mutations in human *SOX9* (fig. 4) do not point to any part of the protein as uniquely important for a role in sex determination.

Expression of *Sox9*

Much has been written about the expression of *Sox9* during chondrogenesis [35, 121, 132, 145], and it is beyond the scope of this review to reiterate these data. Accumulated data from several laboratories is consistent with the notion that *Sox9* serves as an essential trigger for the differentiation of chondrocytes from mesenchymal cells in the embryo, analogous to the proposed role of *Sry* in stimulating Sertoli cell differentiation in mammals.

Expression of *Sox9* during sex determination suggests a role downstream from *Sry* in the Sertoli cell-differentiation pathway. This expression profile has been studied independently by two laboratories, with slightly different outcomes. Lovell-Badge and colleagues reported expression of *Sox9* in mouse genital ridges of both sexes at 10.5 dpc, whereas Koopman and colleagues did not detect *Sox9* transcripts in either sex at the same stage [119, 120]. The discrepancy is almost certainly due to differences in sensitivity in the two separate series of experiments. Both groups, however, reported a strong upregulation of *Sox9* expression in male genital ridges at 11.5 dpc. This appears to coincide with a downregulation of *Sox9* expression in the female. *Sox9* expression is subsequently maintained in testes during and after their differentiation. *Sox9* RNA expression is associated with developing testis cords, and is not due to germ cells, implicating Sertoli cells as the site of *Sox9* expression [119]. Sertoli cell-specific expression of SOX9 protein has been confirmed by immunohistochemistry [120, 146]. *Sox9* expression in Sertoli cells is not transient, but persists through to adulthood.

As with SRY, levels of functional SOX9 protein appear to be critical for male sex determination. Sex reversal associated with CD results from mutation of one copy of *SOX9*. As mentioned above, some of these mutations are likely to impair the ability of the SOX9 protein in an affected individual to bind to DNA. Other mutations would be expected to have no effect on DNA binding, but instead impair other biochemical functions of the protein such as transcriptional transactivation [140]. A third class of mutations is represented by translocation breakpoints upstream of *SOX9* [134, 147]; these would presumably reduce or abolish transcription from the mutant *SOX9* allele.

Sry, *Sox9* and the testis-determining pathway

How do *Sry* and *Sox9* interconnect with each other and with the other pieces of the sex-determination jigsaw identified to date? Several reviews have speculated on the nature of the mammalian sex-determining pathway [148–151]. The following discussion summarizes the current issues and pertinent observations relating to *Sry*, *Sox9* and their interrelationship in the context of sex determination. These relationships are schematically represented in figure 3.

What regulates *Sry*?

WT1, SF1 and LIM1 are implicated in the regulation of *Sry* expression by virtue of their early expression in the genital ridges, but it is also likely that these factors may be involved in establishing an appropriate tissue or biochemical environment for the action of *Sry*, given their knockout phenotypes. The sex reversal seen when *M. m. musculus Sry* alleles are present on an *M. m. domesticus* background implicates aberrant interaction between *musculus Sry* and *domesticus* autosomal/X-linked genes [66], and these genes are likely to be regulators of *Sry* transcription [152, 153] (C. Nagamine et al., unpublished analysis). Some of these loci have been mapped [154], and await positional cloning.

Does SRY directly regulate *Sox9*?

The timing and male-specific upregulation of *Sox9* expression in mice are compatible with the possibility that SRY directly regulates *Sox9*. Indeed, a potential binding site for SRY is found in the promoter region of mouse and human *Sox9*. However, this site is not within a 113-bp interval found to confer at least some sex specificity of *Sox9* expression upon reporter constructs transfected into primary mouse fetal testicular and ovarian cells (Y. Kanai and P. Koopman, unpublished analysis). Even if *Sry* were normally involved in *Sox9* regulation in mammals, evidence exists that it is not absolutely required. In mice, genital ridges of either sex can undergo testicular differentiation when grafted to extragonadal sites such as the kidneys, and testicular tissue induced in this manner expresses *Sox9* [120]. In all, these data do not support direct regulation of *Sox9* by SRY in mammals, although as discussed earlier in this review, caution is required in extrapolating from the situation in mice to that in other mammals.

The role of *Sox9* in sex determination is not limited to mammals. Male-specific expression of *Sox9* has been reported in chicken and turtle genital ridges, coincident with testis determination in those species [119, 120, 155]. This suggests that *Sox9* is a fundamental component of the male sex-determining pathway in all vertebrates. *Sry*

can obviously not be involved in the male-specific expression of *Sox9* in the gonads of nonmammalian species, nor indeed the few mammalian species which lack *Sry*. In classes such as birds, reptiles and fish, different sex-determining switches operate, as described in other papers in this volume. It is generally postulated that sex determination in all vertebrates converges to a common genetic pathway, and that this pathway is activated by different switch mechanisms that can be genetic or temperature-dependent. *Sox9* may represent the point at which these pathways converge. If this is true, then it is possible that this gene is activated by different mechanisms in different vertebrate classes. Alternatively, the pathways may converge at some point upstream from *Sox9*, and similar regulatory mechanisms for *Sox9* might be found in all vertebrates.

Clearly, *Sox9* is a very early-acting gene in the male sex-determination pathway. Mutation of *SOX9* can result in complete, male-to-female phenotypic sex reversal in humans. It appears that *Sox9* is intimately linked with Sertoli cell differentiation, even in the absence of *Sry* [120]. To further test a proposed causal relationship between *Sox9* expression and Sertoli cell differentiation, it will be necessary to make transgenic mice expressing *Sox9* in female genital ridges, and assay for sex reversal in a manner analogous to transgenic studies involving *Sry*.

SRY, DAX1 and the repression of *Sox9*

The expression profiles of *Sox9* in male and female gonads suggests that repression of *Sox9* is critical for ovarian development. It is possible to speculate that a repressor of *Sox9* may be expressed in female genital ridges, but that this repressor is itself repressed in male genital ridges, perhaps by *Sry*. As noted above, transgenic mice overexpressing *Dax1* have been shown to undergo male-to-female sex reversal [100]. This situation is analogous to duplications of the region including *DAX1*, resulting in male-to-female sex reversal in humans [96], although in this latter case the involvement of alternative or additional genes has not been ruled out. Unlike the situation in humans, however, sex reversal in mice was seen only in combination with a 'weak' allele of *Sry* such as Y^{POS}, suggesting that dosage sensitivities differ between the two species. These transgenic experiments suggest that *Dax1* antagonizes *Sry* action [100]. One might speculate that *Dax1* is involved in the suppression of *Sox9*, and that *Sry*'s role is to antagonize *Dax1*. This scenario is really a more refined version of the '*Sry* represses a repressor of maleness' hypothesis of McElreavey and colleagues [31], and it will be of interest to clarify the molecular basis of the antagonism between SRY and DAX1.

Regulation of *Sox9*

While many alternative models are possible, further clarification of the connection between *Sry* and *Sox9* awaits characterization of the *Sox9* regulatory sequences. As mentioned above, we have made some progress towards this end in mice, implicating sequences in the proximal promoter region as contributing, at least in part, to the sex specificity of *Sox9* expression. It appears that other important elements are scattered for some distance upstream of *SOX9*. CD can be caused by translocations involving breakpoints spread through more than 800 kb upstream from *SOX9* [147, 156, 157]. Wunderle and colleagues [158] have delineated some of the regulatory regions using human *SOX9* YAC-LacZ reporter constructs in transgenic mice. While their results indicate that elements required for *SOX9* expression in a variety of tissues are spread through some 350 kb upstream of the gene, they were unable to identify any fragment which directed LacZ expression to the gonads. The sex-specific regulatory elements either are not located in the YAC fragments tested, or do not function in mouse cells. If the latter is true, this may once again point to fundamental biochemical differences in the sex-determining pathway between mice and humans.

Beyond *Sox9*

What lies downstream from *Sox9* in the testis-determining pathway? One obvious candidate is *Amh*. It has been shown that SF1 is important for the regulation of the *Amh* gene: binding sites for SF1 exist in the *Amh* critical regulatory region, and mutation of these abolishes AMH expression in transgenic mice [90, 91]. More recently, SOX9 has been shown to cooperate with SF1 in the regulation of *Amh*. The two proteins bind to adjacent sites in the *Amh* promoter, were shown to interact in a mammalian two-hybrid assay and synergize in trans-activation of an *Amh*-CAT reporter when cotransfected into NT2/D1 human embryonal carcinoma cells [159].

Several observations complicate this simple view that the role of *Sox9* in sex determination is to regulate *Amh*. Expression of *Amh* has been found to precede that of *Sox9* in chickens, not vice versa, which would appear to rule out the possibility of *Amh* regulation by SOX9 in that species [160]. Further, other regulators of *Amh* have been identified in recent studies. It has been reported that SF1 associates with two of the four alternately spliced isoforms of WT1, and that this association synergizes the upregulation of *Amh* [161]. WT1 missense mutations associated with male pseudohermaphroditism fail to synergize with SF1 [161]. The transcription factor GATA-4 has also been identified as a regulator of *Amh* expression in vitro [162]. Perhaps

most important, SOX9 must have other regulatory targets, since *SOX9* mutation results in sex reversal [129, 130], whereas *Amh* mutation does not [86].

An obvious possible target of SOX9 is *SF1*. Potential SOX9 binding sites are found upstream of the *SF1* gene [163], but no direct evidence of a regulatory relationship has been put forward.

Perspectives

Sex determination is fundamental to the survival of any sexually reproducing species. Altering any one of the genetic steps leading to maleness or femaleness would seem to call for simultaneously altering the interdependent steps if that change is to be compatible with normal sexual development, reproduction and hence survival. With this in mind it is indeed astonishing that so many different mechanisms of determining sex can be found among metazoans. The few model organisms studied in any detail provide what is probably only a glimpse of this variability. For example, the pathway used in *Drosophila melanogaster* uses a completely different set of genes to those used in *Caenorhabditis elegans* [164]. None of the genes used in either of these species is deployed in vertebrate sex determination, with the possible exception of *DMT1*, a human gene related to *Drosophila doublesex* and *C. elegans mab3* [165]. This is in stark contrast to many other genetic pathways, such as the *Hox*, *Wingless/Wnt* and *hedgehog* pathways, that have been structurally and functionally conserved throughout the metazoan subkingdom and perhaps even beyond.

In trying to draw together data relating to the biochemical and genetic roles of *Sry* and *Sox9* in sex determination, we have seen several examples of differences between vertebrate classes, mammalian genera, rodent species and in some cases even mouse strains, to a degree that far exceeds all other organogenetic systems. To recount just a few examples, there are differences in dosage sensitivities and gene expression patterns, renegade rodents that lack *Sry* altogether, and a panoply of different structural variations of *Sry* among mice. These differences demand that we resist the temptation to put forward unifying models of the genetic control of sex determination. A corollary is that any individual organism will be of only limited value as a 'model' of vertebrate or mammalian sex determination. Further study of a number of experimental organisms will undoubtedly reveal further richness of variety.

While sex determination in vertebrates is often described as a pathway, perhaps by analogy with the linear sequence of events in *Drosophila* and *C. elegans*, it is clear that this concept is hopelessly inadequate. Several distinct cell lineages need to be coordinately directed by a series of signalling molecules, receptors, signal transduction systems and transcription factors. A number of factors, such as WT1 and SF1, are clearly used several times in different roles, even within the developing gonad. Recent work has begun to uncover the combinatorial transcription factor interactions involved in various aspects of gonadal development. Clearly, we are dealing with a complex network involving multiple positive and negative regulatory elements. *Sry* and *Sox9* play pivotal roles within this network, but information relating to their modes of action remains scant. To date, the majority of efforts to understand the interplay of genes and gene products in sex determination have revolved around attempts at pairwise marriages—SRY and *Amh*, SOX9 and SF1, SOX9 and *Amh* and so on. Some of these relationships are no doubt genuine. However, as described above and elsewhere in this volume, some of these studies have resulted in alliances that are likely to be artefactual; others are contradictory or mutually difficult to reconcile. Part of the reason for this is the molecular biologist's reliance on in vitro systems, which, while useful, may not always reflect the in vivo situation. Another factor is our natural tendency to fit together the pieces of the jigsaw that we have, without regard for the missing pieces. Since we have little more than a handful of transcription factors, it is clear that many more molecules of different classes need to be found. This, and the interconnection of these pieces, will provide the challenge for future research.

Acknowledgements. I am grateful to Gerd Scherer, Andrew Sinclair, Robin Lovell-Badge, Patrick Tam and Vince Harley for helpful comments on the manuscript, and to Jo Bowles, Yoshiakira Kanai, Claude Nagamine and Vince Harley for permission to cite unpublished data. This work was supported by grants from the Australian Research Council and the National Health and Medical Research Council of Australia. P.K. is an Australian Research Council Senior Research Fellow.

1 Ford C. E., Jones K. W., Polani P. E., de Almeida J. C. and Briggs J. H. (1959) A sex chromosome anomaly in a case of gonadal dysgenesis (Turner's Syndrome). Lancet **i**: 711–713
2 Welshons W. J. and Russell L. B. (1959) The Y chromosome as the bearer of male determining factors in the mouse. Proc. Natl. Acad. Sci. USA **45:** 560–566
3 Jacobs P. A. and Strong J. A. (1959) A case of human intersexuality having a possible XXY sex determining mechanism. Nature **183:** 302–303
4 Ferguson-Smith M. A. (1966) X-Y interchange in the aetiology of true hermaphroditism and of XX Klinefelter's syndrome. Lancet **ii**: 475–476
5 Ferguson-Smith M. A. and Affara N. A. (1988) Accidental X-Y recombination and the aetiology of XX males and true hermaphrodites. Philos. Trans. R. Soc. Lond. Biol. **322:** 133–144
6 Sinclair A. H., Berta P., Palmer M. S., Hawkins J. R., Griffiths B. L., Smith M. J. et al. (1990) A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature **346:** 240–244

7 Gubbay J., Collignon J., Koopman P., Capel B., Economou A., Münsterberg A. et al. (1990) A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. Nature **346:** 245–250
8 Berta P., Hawkins J. R., Sinclair A. H., Taylor A., Griffiths B. L., Goodfellow P. N. et al. (1990) Genetic evidence equating *SRY* and the male sex determining gene. Nature **348:** 448–450
9 Jäger R. J., Anvret M., Hall K. and Scherer G. (1990) A human XY female with a frame shift mutation in the candidate testis-determining gene *SRY*. Nature **348:** 452–454
10 Koopman P., Gubbay J., Vivian N., Goodfellow P. and Lovell-Badge R. (1991) Male development of chromosomally female mice transgenic for *Sry*. Nature **351:** 117–121
11 Jantzen H.-M., Admon A., Bell S. P. and Tjian R. (1990) Nucleolar transcription factor hUBF contains a DNA-binding motif with homology to HMG proteins. Nature **344:** 830–836
12 van de Wetering M., Oosterwegel M., Dooijes D. and Clevers H. (1991) Identification and cloning of TCF-1, a T lymphocyte-specific transcription factor containing a sequence-specific HMG box. EMBO J. **10:** 123–132
13 Oosterwegel M., van de Wetering M., Dooijes D., Klomp L., Winoto A., Georgopoulos K. et al. (1991) Cloning of murine TCF-1, a T cell-specific transcription factor interacting with functional motifs in the CD3-epsilon and T cell receptor alpha enhancers. J. Exp. Med. **173:** 1133–1142
14 Travis A., Amsterdam A., Belanger C. and Grosschedl R. (1991) LEF-1, a gene encoding a lymphoid-specific protein with an HMG domain, regulates T-cell receptor α enhancer function. Genes Dev. **5:** 880–894
15 Waterman M. L., Fischer W. H. and Jones K. A. (1991) A thymus-specific member of the HMG protein family regulates the human T cell receptor Cα enhancer. Genes Dev. **5:** 656–669
16 Harley V. R., Jackson D. I., Hextall P. J., Hawkins J. R., Berkovitz G. D., Sockanathan S. et al. (1992) DNA binding activity of recombinant SRY from normal males and XY females. Science **255:** 453–456
17 van de Wetering M. and Clevers H. (1992) Sequence-specific interaction of the HMG box proteins TCF-1 and SRY occurs within the minor groove of a Watson-Crick double helix. EMBO J. **11:** 3039–3044
18 Harley V. R., Lovell-Badge R. and Goodfellow P. N. (1994) Definition of a concensus DNA binding site for SRY. Nucleic Acids Res. **22:** 1500–1501
19 Ferrari S., Harley V. R., Pontiggia A., Goodfellow P. N. and Lovell-Badge R. (1992) SRY, like HMG1, recognizes sharp angles in DNA. EMBO J. **11:** 4497–4506
20 Giese K., Cox J. and Grosschedl R. (1992) The HMG domain of lymphoid enhancer factor 1 bends DNA and facilitates assembly of functional nucleoprotein structures. Cell **69:** 185–195
21 Giese K., Pagel J. and Grosschedl R. (1994) Distinct DNA-binding properties of the high mobility group domain of murine and human SRY sex-determining factors. Proc. Natl. Acad. Sci. USA **91:** 3368–3374
22 Grosschedl R., Giese J. and Pagel J. (1994) HMG domain proteins: architectural elements in the assembly of nucleoprotein structures. Trends Genet. **10:** 94–100
23 Wolffe A. P. (1994) Architectural transcription factors. Science **264:** 1100–1101
24 Giese K., Kingsley C., Kirshner J. R. and Grosschedl R. (1995) Assembly and function of a TCRα enhancer complex is dependent on LEF-1-induced DNA bending and multiple protein-protein interactions. Genes Dev. **9:** 995–1008
25 Pontiggia A., Rimini R., Harley V. R., Goodfellow P. N., Lovell-Badge R. and Bianchi M. E. (1994) Sex-reversing mutations affect the architecture of SRY-DNA complexes. EMBO J. **13:** 6115–6124
26 Whitfield L. S., Lovell-Badge R. and Goodfellow P. N. (1993) Rapid sequence evolution of the mammalian sex determining gene *SRY*. Nature **364:** 713–715
27 Pamilo P. and O'Neill R. J. W. (1997) Evolution of the Sry genes. Mol. Biol. Evol. **14:** 49–55
28 de la Chapelle A. (1981) The etiology of maleness in XX men. Hum. Genet. **58:** 105–116
29 Numabe H., Nagafuchi S., Nakahori Y., Tamura T., Kiuchi H., Namiki M. et al. (1992) DNA analyses of XX and XX-hypospadiac males. Hum. Genet. **90:** 211–214
30 Fechner P. Y., Marcantonio S. M., Jaswaney V., Stetten G., Goodfellow P. N., Migeon C. J. et al. (1993) The role of the sex determining region Y gene in the etiology of 46,XX maleness. J. Clin. Endocrinol. Metab. **76:** 690–695
31 McElreavey K., Vilain E., Herskowitz I. and Fellous M. (1993) A regulatory cascade hypothesis for mammalian sex determination: SRY represses a negative regulator of male development. Proc. Natl. Acad. Sci. USA **90:** 3368–3372
32 Desclozeaux M., Poulat F., Barbara P. D., Capony J. P., Turowski P., Jay P. et al. (1998) Phosphorylation of an N-Terminal motif enhances DNA-binding activity of the human SRY protein. J. Biol. Chem. **273:** 7988–7995
33 Poulat F., Girard F., Chevron M.-P., Goze C., Rebillard X., Calas B. et al. (1995) Nuclear localization of the testis determining gene product SRY. J. Cell Biol. **128:** 737–748
34 Südbeck P. and Scherer G. (1997) Two independent nuclear localization signals are present in the DNA-binding high mobility group domains of *SRY* and *SOX9*. J. Biol. Chem. **272:** 27848–27852
35 Ng L.-J., Wheatley S., Muscat G. E. O., Conway-Campbell J., Bowles J., Wright E. et al. (1997) SOX9 binds DNA, activates transcription and co-expresses with type II collagen during chondrogenesis in the mouse. Dev. Biol. **183:** 108–121
36 Kamachi Y., Cheah K. S. and Kondoh H. (1999) The mechanism of regulatory target selection by the SOX HMG domain proteins as revealed by comparison of SOX1/2 and SOX9. Mol. Cell. Biol. **19:** 107–120
37 Poulat F., de Santa Barbara P., Desclozeaux M., Soullier S., Moniot B., Bonneaud N. et al. (1997) The human testis determining factor *SRY* binds a nuclear factor containing PDZ protein interaction domains. J. Biol. Chem. **272:** 7167–7172
38 Tajima T., Nakae J., Shinohara N. and Fujieda K. (1994) A novel mutation localized in the 3′ non-HMG box region of the SRY gene in 46,XY gonadal dysgenesis. Human Mol. Genet. **3:** 1187–1189
39 Harley V. R., Lovell-Badge R., Goodfellow P. N. and Hextall P. J. (1996) The HMG box of SRY is a calmodulin binding domain. FEBS Lett. **391:** 24–28
40 Gubbay J., Vivian N., Economou A., Jackson D., Goodfellow P. and Lovell-Badge R. (1992) Inverted repeat structure of the Sry locus in mice. Proc. Natl. Acad. Sci. USA **89:** 7953–7957
41 Dubin R. A. and Ostrer H. (1994) Sry is a transcriptional activator. Mol. Endocrinol. **8:** 1182–1192
42 Coward P., Nagai K., Chen D., Thomas H. D., Nagamine C. M. and Lau Y.-F. C. (1994) Polymorphism of a CAG repeat within *Sry* correlates with B6.Y^{Dom} sex reversal. Nature Genet. **6:** 245–250
43 Dubin R. A., Coward P., Lau Y.-F. C. and Ostrer H. (1995) Functional comparison of the *Mus musculus molossinus* and *Mus musculus domesticus Sry* genes. Mol. Endocrinol. **9:** 1645–1654
44 Lau Y. F. C. and Zhang J. (1998) Sry interactive protein – implication for the mechanisms of sex determination. Cytogenet. Cell Genet. **80:** 128–132
45 Koopman P., Münsterberg A., Capel B., Vivian N. and Lovell-Badge R. (1990) Expression of a candidate sex-determining gene during mouse testis differentiation. Nature **348:** 450–452
46 Hacker A., Capel B., Goodfellow P. and Lovell-Badge R. (1995) Expression of *Sry*, the mouse sex determining gene. Development **121:** 1603–1614
47 Jeske Y. W. A., Mishina Y., Cohen D. R., Behringer R. R. and Koopman P. (1996) Analysis of the role of Amh and

Fra1 in the Sry regulatory pathway. Mol. Reprod. Dev. **44:** 153–158

48 Jeske Y. W. A., Bowles J., Greenfield A. and Koopman P. (1995) Expression of a linear *Sry* transcript in the mouse genital ridge. Nature Genet. **10:** 480–482

49 Zwingman T., Erickson R. P., Boyer T. and Ao A. (1993) Transcription of the sex-determining region genes *Sry* and *Zfy* in the mouse preimplantation embryo. Proc. Natl. Acad. Sci. USA **90:** 814–817

50 Gutierrez-Adan A., Behboodi E., Murray J. D. and Anderson G. B. (1997) Early transcription of the SRY gene by bovine preimplantation embryos. Mol. Reprod. Dev. **48:** 246–250

51 Fiddler M., Abdel-Rahman B., Rappolee D. A. and Pergament E. (1995) Expression of SRY transcripts in preimplantation human embryos. Am. J. Med. Genet. **55:** 80–84

52 Harry J. L., Koopman P., Brennan F. E., Graves J. A. M. and Renfree M. B. (1995) Widespread expression of the testis-determining gene SRY in a marsupial. Nature Genet. **11:** 347–349

53 Payen E., Pailhoux E., Merhi R. A., Gianquinto L., Kirszenbaum M., Locatelli A. et al. (1996) Characterization of ovine SRY transcript and developmental expression of genes involved in sexual differentiation. Int. J. Dev. Biol. **40:** 567–575

54 Clépet C., Schafer A. J., Sinclair A. H., Palmer M. S., Lovell-Badge R. and Goodfellow P. N. (1993) The human *SRY* transcript. Human Mol. Genet. **2:** 2007–2012

55 Capel B., Swain A., Nicolis S., Hacker A., Walter M., Koopman P. et al. (1993) Circular transcripts of the testis determining gene *Sry* in adult mouse testis. Cell **73:** 1019–1030

56 Dolci S., Grimaldi P., Geremia R., Pesce M. and Rossi P. (1997) Identification of a promoter region generating Sry circular transcripts both in germ cells from male adult mice and in male mouse embryonal gonads. Biol. Reprod. **57:** 1128–1135

57 Dubin R. A., Kazmi M. A. and Ostrer H. (1995) Inverted repeats are necessary for circularization of the mouse testis *Sry* transcript. Gene **167:** 245–248

58 McLaren A. (1988) Somatic and germ-cell sex in mammals. Philos. Trans. R. Soc. Lond. Biol. **322:** 3–9

59 Merchant H. (1975) Rat gonadal and ovarian organogenesis with and without germ cells. An ultrastructural study. Dev. Biol. **44:** 1–21

60 McLaren A. (1985) Relation of germ cell sex to gonadal development. In: The Origin and Evolution of Sex, pp. 289–300, Halvorson H. O. and Monroy A. (eds), Liss, New York

61 Burgoyne P. S., Buehr M., Koopman P., Rossant J. and McLaren A. (1988) Cell-autonomous action of the testis-determining gene: Sertoli cells are exclusively XY in XX↔XY chimaeric mouse testes. Development **102:** 443–450

62 Palmer S. J. and Burgoyne P. S. (1991) In situ analysis of fetal, prepuberal and adult XX↔XY chimaeric mouse testes: Sertoli cells are predominantly, but not exclusively, XY. Development **112:** 265–268

63 Rossi P., Dolci S., Albanesi C., Grimaldi P. and Geremia R. (1993) Direct evidence that the mouse sex-determining gene *Sry* is expressed in the somatic cells of male fetal gonads and in the germ cell line in the adult testis. Mol. Reprod. Dev. **34:** 369–373

64 Palmer M. S., Sinclair A. H., Berta P., Ellis N. A., Goodfellow P. N., Abbas N. E. et al. (1989) Genetic evidence that ZFY is not the testis-determining factor. Nature **342:** 937–939

65 Capel B., Rasberry C., Dyson J., Bishop C. E., Simpson E., Vivian N. et al. (1993) Deletion of Y chromosome sequences located outside the testis determining region can cause XY female sex reversal. Nature Genet. **5:** 301–307

66 Eicher E. M., Washburn L. L., Whitney J. B. and Morrow K. E. (1982) *Mus poschiavinus* Y chromosome in the *C57BL/6J* murine genome causes sex reversal. Science **217:** 535–537

67 Veitia R. A., Fellous M. and McElreavey K. (1997) Conservation of Y chromosome-specific sequences immediately 5′ to the testis determining gene in primates. Gene **199:** 63–70

68 Margarit E., Guillen A., Rebordosa C., Vidal-Taboada J., Sanchez M., Ballesta F. et al. (1998) Identification of conserved potentially regulatory sequences of the Sry gene from 10 different species of mammals. Biochem. Biophys. Res. Commun. **245:** 370–377

69 Schmitt-Ney M., Thiele H., Kaltwasser P., Bardoni B., Cisternino M. and Scherer G. (1995) Two novel SRY missense mutations reducing DNA binding identified in XY females and their mosaic fathers. Am. J. Hum. Genet. **56:** 862–869

70 Kwok C., Tyler-Smith C., Mendonca B. B., Hughes I., Berkovitz G. D., Goodfellow P. N. et al. (1996) Mutation analysis of the 2 kb 5′ to SRY in XY females and XY intersex subjects. J. Med. Genet. **33:** 465–468

71 Veitia R., Ion A., Barbaux S., Jobling M. A., Souleyreau N., Ennis K. et al. (1997) Mutations and sequence variants in the testis-determining region of the Y chromosome in individuals with a 46,XY female phenotype. Hum. Genet. **99:** 648–652

72 Poulat F., Desclozeaux M., Tuffery S., Jay P., Boizet B. and Berta P. (1998) Mutation in the 5′ noncoding region of the SRY gene in an XY sex-reversed patient. Hum. Mut. S192–S194

73 McElreavey K., Vilain E., Abbas N., Costa J. M., Souleyreau N., Kucheria K. et al. (1992) XY sex reversal associated with a deletion 5′ to the *SRY* 'HMG box' in the testis-determining region. Proc. Natl. Acad. Sci. USA **89:** 11016–11020

74 McElreavey K., Vilain E., Barbaux S., Fuqua J. S., Fechner P. Y., Souleyreau N. et al. (1996) Loss of sequences 3′ to the testis-determining gene, *SRY*, including the Y pseudoautosomal boundary associated with partial testicular determination. Proc. Natl. Acad. Sci. USA **93:** 8590–8594

75 Griffiths R. (1991) The isolation of conserved DNA sequences related to the human sex-determining region Y gene from the lesser black-backed gull (*Larus fuscus*). Philos. Trans. R. Soc. Lond. Biol. **244:** 123–128

76 Tiersch T. R., Mitchell M. J. and Wachtel S. S. (1991) Studies on the phylogenetic conservation of the SRY gene. Hum. Genet. **87:** 571–573

77 Just W., Rau W., Vogul W., Akhverdian M., Fredga K., Graves J. A. M. et al. (1995) Absence of *Sry* in species of the vole *Ellobius*. Nature Genet. **11:** 117–118

78 Soullier S., Hanni C., Catzeflis F., Berta P. and Laudet V. (1998) Male sex determination in the spiny rat *Tokudaia osimensis* (Rodentia: Muridae) is not Sry dependent. Mamm. Genome **9:** 590–5922

79 Stevanovic M., Lovell-Badge R., Collignon J. and Goodfellow P. N. (1993) *SOX3* is an X-linked gene related to *SRY*. Human Mol. Genet. **2:** 2013–2018

80 Foster J. W. and Graves J. A. M. (1994) An SRY-related sequence on the marsupial X chromosome: implications for the evolution of the mammalian testis determining gene. Proc. Natl. Acad. Sci. USA **91:** 1927–1931

81 Collignon J., Sockanathan S., Hacker A., Cohen-Tannoudji M., Norris D., Rastan S. et al. (1996) A comparison of the properties of *Sox-3* with *Sry* and two related genes, *Sox-1* and *Sox-2*. Development **122:** 509–520

82 Uwanogho D., Rex M., Cartwright E. J., Pearl G., Healy C., Scotting P. et al. (1995) Embryonic expression of the chicken Sox2, Sox3 and Sox11 genes suggests an interactive role in neural development. Mech. Dev. **49:** 23–36

83 Penzel R., Oschwald R., Chen Y. L., Tacke L. and Grunz H. (1997) Characterization and early embryonic expression of a neural specific transcription factor xSox3 in *Xenopus laevis*. Int. J. Dev. Biol. **41:** 667–677

84 McBride D., Sang H. and Clinton M. (1997) Expression of *Sry*-related genes in the developing genital ridge/mesonephros of the chick embryo. J. Reprod. Fertil. **109:** 59–63

85 Mumm S., Zucchi I. and Pilia G. (1997) SOX3 gene maps near DXS984 in Xq27.1, within candidate regions for several X-linked disorders. Am. J. Med. Genet. **72:** 376–378
86 Behringer R. R., Finegold M. J. and Cate R. L. (1994) Müllerian-Inhibiting Substance function during mammalian sexual development. Cell **79:** 415–425
87 Ikeda Y., Shen W. H., Ingraham H. A. and Parker K. L. (1994) Developmental expression of mouse steroidogenic factor-1, an essential regulator of the steroid hydroxylases. Mol. Endocrinol. **8:** 654–662
88 Hatano O., Takayama K., Imai T., Waterman M. R., Takakusu A., Omura T. et al. (1994) Sex-dependent expression of a transcription factor, Ad4BP, regulating steroidogenic P-450 genes in the gonads during prenatal and postnatal rat development. Development **120:** 2787–2797
89 Luo X., Ikeda Y. and Parker K. L. (1994) A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. Cell **77:** 481–490
90 Shen W.-H., Moore C. C. D., Ikeda Y., Parker K. L. and Ingraham H. (1994) Nuclear receptor steriodogenic factor 1 regulates the Müllerian inhibiting substance gene: a link to the sex determination cascade. Cell **77:** 651–661
91 Giuili G., Shen W.-H. and Ingraham H. A. (1997) The nuclear receptor SF-1 mediates sexually dimorphic expression of müllerian inhibiting substance, in vivo. Development **124:** 1799–1807
92 Kreidberg J. A., Sariola H., Loring J. M., Maeda M., Pelletier J., Housman D. et al. (1993) WT-1 is required for early kidney development. Cell **74:** 679–691
93 Pelletier J., Bruening W., Kashtan C. E., Mauer S. M., Manivel J. C., Striegel J. E. et al. (1991) Germline mutations in the Wilms' tumor suppressor gene are associated with abnormal urogenital development in Denys-Drash syndrome. Cell **67:** 437–447
94 Behlke M. A., Bogan J. S., Beer-Romero P. and Page D. C. (1993) Evidence that the SRY protein is encoded by a single exon on the human Y chromosome. Genomics **17:** 736–739
95 Shawlot W. and Behringer R. R. (1995) Requirement for *Lim1* in head-organizer function. Nature **374:** 425–430
96 Bardoni B., Zanaria E., Guioli S., Floridia G., Worley K. C., Tonini G. et al. (1994) A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. Nature Genet. **7:** 497–501
97 Muscatelli F., Strom T. M., Walker A. P., Zanaria E., Recan D., Meindl A. et al. (1994) Mutations in the *DAX-1* gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. Nature **372:** 672–676
98 Zanaria E., Muscatelli F., Bardoni B., Strom T. M., Guioli S., Guo W. et al. (1994) An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. Nature **372:** 635–641
99 Swain A., Zanaria E., Hacker A., Lovell-Badge R. and Camerino G. (1996) Mouse *Dax1* expression is consistent with a role in sex determination as well as adrenal and hypothalamus function. Nature Genet. **12:** 404–409
100 Swain A., Narvaez V., Burgoyne P. S., Camerino G. and Lovell-Badge R. (1998) *Dax1* antagonizes *Sry* action in mammalian sex determination. Nature **391:** 761–767
101 Yu R. N., Ito M., Saunders T. L., Camper S. A. and Jameson J. L. (1998) Role of *Ahch* in gonadal development and gametogenesis. Nature Genet. **20:** 353–357
102 Parker K. L. and Schimmer B. P. (1998) *Ahch* and the feminine mystique. Nature Genet. **20:** 318–319
103 Haqq C. M., King C.-Y., Donahoe P. K. and Weiss M. A. (1993) SRY recognizes conserved DNA sites in sex-specific promoters. Proc. Natl. Acad. Sci. USA **90:** 1097–1101
104 Cohen D. R., Sinclair A. H. and McGovern J. D. (1994) Sry protein enhances transcription of Fos-related antigen 1 promoter constructs. Proc. Natl. Acad. Sci. USA **91:** 4372–4376
105 Prior H. M. and Walter M. A. (1996) SOX genes: architects of development. Mol. Med. **2:** 405–412
106 Pevny L. H. and Lovell-Badge R. (1997) Sox genes find their feet. Curr. Opin. Genet. Dev. **7:** 338–344
107 Wright E. M., Snopek B. and Koopman P. (1993) Seven new members of the *Sox* gene family expressed during mouse development. Nucleic Acids Res. **21:** 744
108 Denny P., Swift S., Connor F. and Ashworth A. (1992) An *SRY*-related gene expressed during spermatogenesis in the mouse encodes a sequence-specific DNA-binding protein. EMBO J. **11:** 3705–3712
109 Denny P., Swift S., Brand N., Dabhade N., Barton P. and Ashworth A. (1992) A conserved family of genes related to the testis determining gene, *SRY*. Nucleic Acids Res. **20:** 2887
110 Schilham M. W., van Ejik M., van de Wetering M. and Clevers H. C. (1993) The murine Sox-4 protein is encoded on a single exon. Nucleic Acids Res. **21:** 2009
111 van de Wetering M. and Clevers H. (1993) *Sox15*, a novel member of the murine *Sox* family of HMG box transcription factors. Nucleic Acids Res. **21:** 1669
112 Hosking B. M., Muscat G. E. O., Koopman P. A., Dowhan D. H. and Dunn T. L. (1995) *Trans*-activation and DNA-binding properties of the transcription factor, Sox-18. Nucleic Acids Res. **23:** 2626–2628
113 Connor F., Wright E., Denny P., Koopman P. and Ashworth A. (1995) The *Sry*-related HMG box-containing gene *Sox6* is expressed in the adult testis and developing nervous system of the mouse. Nucleic Acids Res. **23:** 3365–3372
114 Kanai Y., Kanai-Azuma M., Noce T., Saido T. C., Shiroishi T., Hayashi Y. et al. (1996) Identification of two *Sox17* messenger RNA isoforms, with and without the high mobility group box region, and their differential expression in mouse spermatogenesis. J. Cell Biol. **133:** 667–681
115 Hargrave M., Wright E., Kun J., Emery J., Cooper L. and Koopman P. (1997) Expression of the *Sox11* gene in mouse embryos suggests roles in neuronal maturation and epithelio-mesenchymal induction. Dev. Dyn. **210:** 79–86
116 Southard-Smith E. M., Kos L. and Pavan W. J. (1998) *Sox10* mutation distrupts neural crest development in *Dom* Hirschsprung mouse model. Nature Genet. **18:** 60–64
117 Roose J., Korver W., Oving E., Wilson A., Wagenaar G., Markman M. et al. (1998) High expression of the HMG box factor Sox-13 in arterial walls during embryonic development. Nucleic Acids Res. **26:** 469–476
118 Fukada S., Tanaka M., Iwaya M., Nakajima M. and Nagahama Y. (1995) The *Sox* gene family and its expression during embryogenesis in the teleost fish, medaka (*Oryzias latipes*). Dev. Growth Differ. **37:** 379–385
119 Kent J., Wheatley S. C., Andrews J. E., Sinclair A. H. and Koopman P. (1996) A male-specific role for SOX9 in vertebrate sex determination. Development **122:** 2813–2822
120 Morais da Silva S., Hacker A., Harley V., Goodfellow P., Swain A. and Lovell-Badge R. (1996) *Sox9* expression during gonadal development implies a conserved role for the gene in testis differentiation in mammals and birds. Nature Genet. **14:** 62–68
121 Healy C., Uwanogho D. and Sharpe P. T. (1996) Expression of the chicken *Sox9* gene marks the onset of cartilage differentiation. Ann. N. Y. Acad. Sci. **785:** 261–262
122 Rex M., Uwanogho D. A., Orme A., Scotting P. J. and Sharpe P. T. (1997) *cSox21* exhibits a complex and dynamic pattern of transcription during embryonic development of the chick central nervous system. Mech. Dev. **66:** 39–53
123 Rex M., Orme A., Uwanogho D., Tointon K., Wigmore P. M., Sharpe P. T. et al. (1997) Dynamic expression of chicken Sox2 and Sox3 genes in ectoderm induced to form neural tissue. Dev. Dyn. **209:** 323–332
124 Jay P., Sahly I., Goze C., Taviaux S., Poulat F., Couly G. et al. (1997) SOX22 is a new member of the SOX gene family, mainly expressed in human nervous tissue. Human Mol. Genet. **6:** 1069–1077
125 Kuhlbrodt K., Herbarth B., Sock E., Hermans-Borgmeyer I. and Wegner M. (1998) Sox10, a novel transcriptional modulator in glial cells. J. Neurosci. **18:** 237–250
126 Pusch C., Hustert E., Pfeifer D., Südbeck P., Kist R., Roe B. et al. (1998) The SOX10/Sox10 gene from human and mouse

– sequence, expression and transactivation by the encoded HMG domain transcription factor. Hum. Genet. **103:** 115–123

127 Schilham M. W., Oosterwegel M. A., Moerer P., Ya J., de Boer P. A. J., van de Wetering M. et al. (1996) Defects in cardiac outflow tract formation and pro-B-lyphocyte expansion in mice lacking *Sox-4*. Nature **380:** 711–714

128 Nishiguchi S., Wood H., Kondoh H., Lovell-Badge R. and Episkopou V. (1998) Sox1 directly regulates the gamma-crystallin genes and is essential for lens development in mice. Genes Dev. **12:** 776–781

129 Foster J. W., Dominguez-Steglich M. A., Guioli S., Kwok C., Weller P. A., Weissenbach J. et al. (1994) Campomelic dysplasia and autosomal sex reversal caused by mutations in an *SRY*-related gene. Nature **372:** 525–530

130 Wagner T., Wirth J., Meyer J., Zabel B., Held M., Zimmer J. et al. (1994) Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the *SRY*-related gene *SOX9*. Cell **79:** 1111–1120

131 Pingault V., Bondurand N., Kuhlbrodt K., Goerich D. E., PrEhu M.-O., Puliti A. et al. (1998) *SOX10* mutations in patients with Waardenburg-Hirschsprung disease. Nature Genet. **18:** 171–173

132 Wright E., Hargrave M. R., Christiansen J., Cooper L., Kun J., Evans T. et al. (1995) The *Sry*-related gene *Sox-9* is expressed during chondrogenesis in mouse embryos. Nature Genet. **9:** 15–20

133 Houston C. S., Opitz J. M., Spranger J. W., Macpherson R. I., Reed M. H., Gilbert E. F. et al. (1983) The campomelic syndrome: review, report of 17 cases and follow-up on the currently 17-year old boy first reported by Maroteaux et al. in 1971. Am. J. Med. Genet. **15:** 3–28

134 Tommerup N., Schempp W., Mienecke P., Pedersen S., Bolund L., Brandt C. et al. (1993) Assignment of an autosomal sex reversal locus (SRA1) and campomelic dysplasia (CMPD1) to 17q24.3-q25.1. Nature Genet. **4:** 170–174

135 Bell D. M., Leung K. K. H., Wheatley S. C., Ng L. J., Zhou S., Ling K. W. et al. (1997) SOX9 directly regulates the type-II collagen gene. Nature Genet. **16:** 174–178

136 Lefebvre V., Huang W., Harley V. R., Goodfellow P. N. and De Crombrugghe B. (1997) SOX9 is a potent activator of the chondrocyte-specific enhancer of the pro-alpha-1(II) collagen gene. Mol. Cell. Biol. **17:** 2336–2346

137 Kwok C., Weller P., Guioli S., Foster J. W., Mansour S., Zuffardi O. et al. (1995) Mutations in *SOX9*, the gene responsible for campomelic dysplasia and autosomal sex reversal. Am. J. Hum. Genet. **57:** 1028–1036

138 Meyer J., Südbeck P., Held M., Wagner T., Schmitz M. L., Bricarelli F. D. et al. (1997) Mutational analysis of the SOX9 gene in campomelic dysplasia and autosomal sex reversal: lack of genotype/phenotype correlations. Human Mol. Genet. **6:** 91–98

139 Goji K., Nishijima E., Tsugawa C., Nishio H., Pokharel R. and Matsuo M. (1998) Novel missense mutation in the HMG box of SOX9 gene in a Japanese XY male resulted in campomelic dysplasia and severe defect in masculinization. Hum. Mut. Suppl. 1: S114–S116

140 Südbeck P., Schmitz M. L., Bauerle P. A. and Scherer G. (1996) Sex reversal by loss of the C-terminal transactivation domain of human SOX9. Nature Genet. **13:** 230–232

141 Sekiya I., Koopman P., Watanabe H., Ezura Y., Yamada Y. and Noda M. (1997) SOX9 enhances aggrecan gene expression via the promoter region containing a single HMG box sequence in a chondrogenic cell line, TC6. J. Bone Miner. Res. **12:** P222

142 Lefebvre V., Li P. and de Crombrugghe B. (1998) A new long form of Sox5 (L-Sox5), Sox6 and Sox9 are coexpressed in chondrogenesis and cooperatively activate the type II collagen gene. EMBO J. **17:** 5718–5733

143 Schafer A. J., Dominguez-Steglich M. A., Guioli S., Kwok C., Weller P. A., Stevanovic M. et al. (1995) The role of SOX9 in autosomal sex reversal and campomelic dysplasia. Philos. Trans. R. Soc. Lond. Biol. **350:** 271–278

144 Kwok C., Goodfellow P. N. and Hawkins J. R. (1996) Evidence to exclude SOX9 as a candidate gene for XY sex reversal without skeletal malformation. J. Med. Genet. **33:** 800–801

145 Zhao Q., Eberspaecher H., Lefebvre V. and De Crombrugghe B. (1997) Parallel expression of *Sox9* and *Col2al* in cells undergoing chondrogenesis. Dev. Dyn. **209:** 377–386

146 Jamieson R. V., Zhou S. X., Wheatley S. C., Koopman P. and Tam P. P. L. (1998) Sertoli cell differentiation and Y-chromosome activity – a developmental study of X-linked transgene activity in sex-reversed X/XSxr(a) mouse embryos. Dev. Biol. **199:** 235–244

147 Wirth J., Wagner T., Meyer J., Pfeiffer R. A., Tietze H. U., Schempp W. et al. (1996) Translocation breakpoints in three patients with campomelic dysplasia and autosomal sex reversal map more than 130 kb from SOX9. Hum. Genet. **97:** 186–193

148 Goodfellow P. N. and Lovell-Badge R. (1993) *Sry* and sex determination in mammals. Ann. Rev. Genet. **27:** 71–92

149 McElreavey K. and Fellous M. (1997) Sex-determining genes. Trends Endocrinol. Metab. **8:** 342–346

150 Greenfield A. (1998) Genes, cells and organs: recent developments in the molecular genetics of mammalian sex determination. Mamm. Genome **9:** 683–687

151 Capel B. (1998) Sex in the 90s – Sry and the switch to the male pathway. Ann. Rev. Physiol. **60:** 497–523

152 Eicher E. M., Shown E. P. and Washburn L. L. (1995) Sex reversal in C57BL/6J-Y-POS mice corrected by a Sry transgene. Philos. Trans. R. Soc. Lond. Biol. **350:** 263–269

153 Albrecht K. H. and Eicher E. M. (1997) DNA sequence analysis of Sry alleles (subgenus Mus) implicates misregulation as the cause of C57BL/6J-Y-pos sex reversal and defines the Sry functional unit. Genetics **147:** 1267–1277

154 Eicher E. M., Washburn L. L., Schork N. J., Lee B. K., Shown E. P., Xu X. et al. (1996) Sex-determining genes on mouse autosomes identified by linkage analysis of C57BL/6J-Y^{POS} sex reversal. Nature Genet. **14:** 206–209

155 Spotila L. D., Spotila J. R. and Hall S. E. (1998) Sequence and expression analysis of WT1 and Sox9 in the red-eared slider turtle, *Trachemys scripta*. J. Expt. Zool. **281:** 417–427

156 Pfeifer D., Kist R., Meyer J., Zimmer J., Korniszewski L., Stankiewicz P. et al. (1998) Campomelic dysplasia translocation patients have breakpoints scattered over more than 600kb proximal to SOX9. Am. J. Hum. Genet. **61** (Suppl.): A343

157 Scherer G., Kist R., Meyer J., Zimmer J., Korniszewski L., Stankiewicz P. et al. (1998) Campomelic dysplasia translocation patients have breakpoints scattered over more than 800kb proximal to SOX9. Eur. J. Hum. Genet. **6** (Suppl. 1): 115

158 Wunderle V. M., Critcher R., Hastie N., Goodfellow P. N. and Schedl A. (1998) Deletion of long-range regulatory elements upstream of SOX9 causes campomelic dysplasia. Proc. Natl. Acad. Sci. USA **95:** 10649–10654

159 de Santa Barbara P., Bonneaud N., Boizet B., Desclozeaux M., Moniot B., Südbeck P. et al. (1998) Direct interaction of SRY-related protein SOX9 and steroidogenic factor 1 regulates transcription of the human anti-Müllerian hormone gene. Mol. Cell. Biol. **18:** 6653–6665

160 Oreal E., Pieau C., Mattei M. G., Josso N., Picard J. Y., Carré-Eusebe D. et al. (1998) Early expression of Amh in chicken embryonic gonads precedes testicular Sox9 expression. Dev. Dyn. **212:** 522–532

161 Nachtigal M. W., Hirokawa Y., Enyeart-van Houten D. L., Flanagan J. N., Hammer G. D. and Ingraham H. A. (1998) Wilms' tumor 1 and Dax1 modulate the orphan nuclear receptor SF1 in sex-specific gene expression. Cell **93:** 445–454

162 Viger R. S., Mertineit C., Trasler J. M. and Nemer M. (1998) Transcription factor GATA-4 is expressed in a sexually dimorphic pattern during mouse gonadal development and is a potent activator of the Müllerian inhibiting substance promoter. Development **125:** 2665–2675

163 Nomura M., Bartsch S., Nawata H., Omura T. and Morohashi K. (1995) An E box element is required for the expression of the *ad4bp* gene, a mammalian homologue of *ftz-f1* gene, which is essential for adrenal and gonadal development. J. Biol. Chem. **270:** 7453–7461

164 Hodgkin J. (1990) Sex determination compared in *Drosophila* and *Caenorhabditis*. Nature **344:** 721–728

165 Raymond C. S., Shamu C. E., Shen M. M., Seifert K. J., Hirsch B., Hodgkin J. et al. (1998) Evidence for evolutionary conservation of sex-determining genes. Nature **391:** 691–695

166 Werner M. H., Huth J. R., Gronenborn A. M. and Clore G. M. (1995) Molecular basis of human 46 X,Y sex reversal revealed from the three-dimensional solution structure of the human SRY-DNA complex. Cell **81:** 705–714

167 Jäger R., Harley V., Pfeiffer R., Goodfellow P. and Scherer G. (1992) A familial mutation in the testis-determining gene SRY shared by both sexes. Hum. Genet. **90:** 350–355

168 Hawkins J., Taylor A., Goodfellow P., Migeon C., Smith K. and Berkovitz G. (1992) Evidence for increased prevalence of SRY mutations in XY females with complete rather than partial gonadal dysgenesis. Am. J. Hum. Genet. **51:** 979–984

169 Muller J., Schwartz M. and Skakkebaek N. (1992) Analysis of the sex-determining region of the Y chromosome (SRY) in sex reversed patients: point-mutation in SRY causing sex-reversion in a 46,XY female. J. Clin. Endocrinol. Metab. **75:** 331–333

170 McElreavey K. D., Vilain E., Boucekkine C., Vidaud M., Jaubert F., Richaud F. et al. (1992) XY sex reversal associated with a nonsense mutation in SRY. Genomics **13:** 838–840

171 Zeng Y., Ren Z., Zhang M., Huang Y., Zeng F. and Huang S. (1993) A new de novo mutation (A113T) in HMG box of the SRY gene leads to XY gonadal dysgenesis. J. Med. Genet. **30:** 655–657

172 Affara N. A., Chalmers I. J. and Ferguson-Smith M. A. (1993) Analysis of the SRY gene in 22 sex-reversed XY females identifies four new point mutations in the conserved DNA binding domain. Human Mol. Genet. **2:** 785–789

173 Braun A., Kammerer S., Cleve H., Lohrs U., Schwarz H. and Kuhnle U. (1993) True hermaphroditism in a 46,XY individual, caused by a postzygotic somatic point mutation in the male gonadal sex-determining locus (SRY): molecular genetics and histological findings in a sporadic case. Am. J. Hum. Genet. **52:** 578–585

174 Poulat F., Soullier S., Gozé C., Heitz F., Calas B. and Berta P. (1994) Description and functional implications of a novel mutation in the sex-determining gene SRY. Hum. Mut. **3:** 200–204

175 Iida T., Nakahori Y., Komaki R., Mori E., Hayashi N., Tsutsumi O. et al. (1994) A novel nonsense mutation in the HMG box of the SRY gene in a patient with XY sex reversal. Human Mol. Genet. **3:** 1437–1438

176 Hiort O., Gramss B. and Klauber G. (1995) True hermaphroditism with 46,XY karyotype and a point mutation in the SRY gene. J. Pediatr. **126:** 1022

177 Bilbao J. R., Loridan L. and Castano L. (1996) Novel postzygotic nonsense mutation in SRY in familial XY gonadal dysgenesis. Hum. Genet. **97:** 537–539

178 Battiloro E., Angeletti B., Tozzi M. C., Bruni L., Tondini S., Vignetti P. et al. (1997) A novel double nucleotide substitution in the HMG box of the SRY gene associated with Swyer syndrome. Hum. Genet. **100:** 585–587

179 Hines R. S., Tho S. P. T., Zhang Y. Y., Plouffe L., Jr, Hansen K. A., Khan I. et al. (1997) Paternal somatic and germ-line mosaicism for a sex-determining region on Y (SRY) missense mutation leading to recurrent 46,XY sex reversal. Fertil. Steril. **67**: 675–679

180 Cameron F. J., Smith M. J., Warne G. L. and Sinclair A. H. (1998) Novel mutation in the Sry gene results in 46,XY gonadal dysgenesis. Hum. Mut. Suppl. 1: S110–S111

181 Scherer G., Held M., Erdel M., Meschede D., Horst J., Lesniewicz R. et al. (1998) Three novel SRY mutations in XY gonadal dysgenesis and the enigma of XY gonadal dysgenesis cases without SRY mutations. Cytogenet. Cell Genet. **80:** 188–192

182 Domenice S., Yumie Nishi M., Correia Billerbeck A., Latronico A., Aparecida Medeiros M., Russell A. et al. (1998) A novel missense mutation (S18N) in the 5′ non-HMG box region of the SRY gene in a patient with partial gonadal dysgenesis and his normal male relatives. Hum. Genet. **102:** 213–215

183 Brown S., Yu C., Lanzano P., Heller D., Thomas L., Warburton D. et al. (1998) A de novo mutation (Gln2Stop) at the 5′ end of the SRY gene leads to sex reversal with partial ovarian function. Am. J. Hum. Genet. **62:** 189–192

184 Dörk T., Stuhrmann M., Miller K. and Schmidtke J. (1998) Independent observation of SRY mutation I90M in a patient with complete gonadal dysgenesis. Hum. Mut. **11:** 90–91

185 Cameron F. J., Hageman R. M., Cooke-Yarborough C., Kwok C., Goodwin L. L., Sillence D. O. et al. (1996) A novel germ line mutation in *SOX9* causes familial campomelic dysplasia and sex reversal. Human Mol. Genet. **5:** 1625–1630

186 Hageman R. M., Cameron F. J. and Sinclair A. H. (1998) Mutation analysis of the *SOX9* gene in a patient with campomelic dysplasia. Hum. Mut. Suppl. 1: S112–S113

187 Takamatsu N., Kanda H., Ito M., Yamashita A., Yamashita S. and Shiba T. (1997) Rainbow trout SOX9: cDNA cloning, gene structure and expression. Gene **202:** 167–170