Sex Determination

- History
- Jost model of sexual differentiation
  - Chromosomal sex
  - Gonadal sex
  - Phenotypic sex
- Gonadal development systems
  - Cell biology
  - Required genes
- How does chromosomal sex dictate gonadal sex?
  - Molecular cloning of testis-determining factor(s) (e.g. SRY)
  - Interactions of SRY and SOX genes
  - X chromosome sex determining factor DSS/DAX
  - Interactions SRY, SOX, DAX, SF1, and DMRT
- How does gonadal sex dictate phenotypic sex?
  - Müllerian Inhibitory Substance (MIS)
  - Androgen induced male differentiation
- Abnormal sexual differentiation
  - New potential sex determination genes
- Mechanisms of sex determination in other species

Required Reading


References


that are more credible than currently available projections.

Beata M. Csatho is in the Department of Geological Sciences, University at Buffalo, New York 14260, USA.


In retrospect

Twenty-five years of the sex-determining gene

The discovery that the gene SRY on the mammalian Y chromosome drives testis development marked a turning point in the decades-long quest to understand the genetic underpinnings and evolution of sex determination.

JENNIFER A. MARSHALL GRAVES

It has long been known that a testis-determining factor (TDF) on the Y chromosome kick-starts testis development in humans and other mammals. The testes make hormones, and these hormones make the embryo male. Twenty-five years ago, Sinclair et al. reported in Nature that TDF was the gene SRY. This discovery opened up the surprisingly intricate genetic pathway that determines whether a baby is born a boy or a girl. It also led to an understanding of how genes on the Y chromosome evolved, and of the impact of this key evolutionary event.

Until the 1980s, there was no viable candidate sex-determining gene. Just where was TDF located? What kind of product did it encode? What did it do? During the 1980s, the position of TDF was narrowed down to a small region on the short arm of the Y chromosome, when it was found that some males had XX chromosomes that harboured a small piece of the Y, whereas some females had XY chromosomes that lacked bits of the Y — these added and deleted regions of Y were assumed to contain the TDF sequence. The race was then on to find TDF.

In 1987, the geneticist David Page and his associates identified the first coding gene on the human Y, called ZFY. The gene looked like a winning candidate: it was in the right place; it was expressed in the testis; and it was evolutionarily conserved in other placental mammals, such as monkeys, mice, dogs and horses. But in 1988, PhD students in my laboratory, Andrew Sinclair and Jamie Foster, mapped ZFY to a non-sex chromosome (an autosome) in marsupials, which are a separate branch of mammals. A few months later, it was found that, although ZFY is expressed in mouse sperm precursors, it is absent from the other cells of the testis, where a true TDF must be expressed to exert a sex-determining effect.

Sinclair joined a renewed hunt for human TDF in the laboratory of geneticist Peter Goodfellow, using DNA from XY males that had even smaller pieces of the Y than had previously been studied. This was slow and frustrating work, because the Y chromosome is full of repetitive sequences and so specific regions are hard to pinpoint. It was 1990 before they found a small coding gene close to the end of the Y chromosome (Fig. 1). Noncommittally they called the gene SRY, for sex region on the Y. The final proof that SRY was the TDF came from the discovery of SRY mutations in XY females and from the demonstration that adding Sry to XX mice was sufficient to induce male development. SRY was located on the Y in other placental mammals and, thankfully, even in marsupials.

Researchers in the field imagined that identifying TDF would rapidly lead to an understanding of how it worked, and would point to other genes in the sex-determining pathway. But 25 years on, it has become clear that the pathway kick-started by SRY is complex, full of checks and balances.

Initially, SRY proved a puzzle because it was unlike any known gene. It turned out to be a member of a previously unidentified family, now called the SOX genes. Painstaking biochemical studies of the SRY protein revealed that it bound to a certain DNA sequence and bent it at an angle, presumably to bring other sequences — or the proteins bound to them — into proximity, promoting or inhibiting transcription. The discovery of a different...
SOX gene that was disrupted in XY female babies with a severe bone deformity, revealed that this gene, SOX9, is the binding target of SRY protein. SOX9 is now known to be a master regulator of sex determination throughout the vertebrates.

Studying the mutations that cause sex reversal in humans, mice, goats or dogs (the same pathway is active in all mammals) has proved a successful strategy for identifying many genes in the sex-determination pathway. Gradually, a network of genes that are regulated by, or regulate, SRY or SOX9 has been constructed, and their function tested by mutating the genes in mice. Some genes promote testis formation, some maintain it, and yet others oppose them. This pathway and its control is still being explored. Our improved understanding has helped us both to answer fundamental scientific questions and to diagnose and treat many babies who are born with disorders of sex determination.

The other major line of research enabled by the identification of SRY was the evolution of sex genes and chromosomes. The hunt for SRY in marsupials revealed that mammals have an SRY-related gene on the X chromosome, SOX3, which was proposed to be the ancestor of SRY. This idea is supported by human and mouse data that showed that misexpression of SOX3 in the undifferentiated gonad (a tissue can develop into either an ovary or a testis, depending on the signals it receives) drives male development in XX embryos. SRY probably evolved from SOX3 when its S′ region was replaced by a promoter sequence that drove expression in the gonad (Fig. 1).

Although it might seem counterintuitive that the testis-determining factor evolved from the X chromosome, it has since emerged that 20 of the 27 genes on the male-specific part of the human Y evolved from genes on the X. Thus, the Y is basically a degraded X chromosome. This supports the hypothesis that sex chromosomes originate when one member of an autosome pair acquires a sex-determining gene. Nearby genes then also acquire a sex-specific function, crossing over between the chromosome pair is suppressed to keep the male-specific gene package together, and the genetically isolated region on the sex-specific chromosome degrades rapidly.

The mammalian XY sex pair was probably defined by the evolution of SRY. Vertebrate phylogeny puts the age of SRY and the XY pair at between 166 million and 190 million years old. Furthermore, rapid speciation in other lineages that have undergone sex-chromosome turnover raises the possibility that acquisition of SRY might have driven the divergence of the eg-lying monotreme mammals from the rest of the mammalian lineage — monotremes have a bizarre, complex sex-determination system that is related to bird sex chromosomes.

The future of the Y chromosome is now hotly debated. Evidence suggests that the mammalian Y will disappear in just a few million years if gene loss continues at the same rate as in the past. It has already disappeared in two groups of rodents, and SRY has been replaced by another gene from the sex-determining network. The primate Y seems more stable, but will eventually erode away. Humans may be in for another round of sex-chromosome turnover — and maybe speciation — if and when SRY finally bows out.

Jennifer A. Marshall Graves is at the School of Life Science, La Trobe University, Melbourne, Victoria 3086, Australia, and at the Research School of Biology, Australian National University, Canberra.

e-mail: j.graves@latrobe.edu.au

Plasticity of gene-regulatory networks controlling sex determination: of masters, slaves, usual suspects, newcomers, and usurpators

Amaury Herpin & Manfred Schartl

Abstract

Sexual dimorphism is one of the most pervasive and diverse features of animal morphology, physiology, and behavior. Despite the generality of the phenomenon itself, the mechanisms controlling how sex is determined differ considerably among various organismic groups, have evolved repeatedly and independently, and the underlying molecular pathways can change quickly during evolution. Even within closely related groups of organisms for which the development of gonads on the morphological, histological, and cell biological level is indistinguishable, the molecular control and the regulation of the factors involved in sex determination and gonad differentiation can be substantially different. The biological meaning of the high molecular plasticity of an otherwise common developmental program is unknown. While comparative studies suggest that the downstream effectors of sex-determining pathways tend to be more stable than the triggering mechanisms at the top, it is still unclear how conserved the downstream networks are and how all components work together. After many years of stasis, when the molecular basis of sex determination was amenable only in the few classical model organisms (fly, worm, mouse), recently, sex-determining genes from several animal species have been identified and new studies have elucidated some novel regulatory interactions and biological functions of the downstream network, particularly in vertebrates. These data have considerably changed our classical perception of a simple linear developmental cascade that makes the decision for the embryo to develop as male or female, and how it evolves.

Keywords

Dmrt; ovary; SRY; testis; transcription factor

DOI 10.15252/embr.201540667 | Received 13 May 2015 | Revised 28 July 2015 | Accepted 31 July 2015 | Published online 10 September 2015


See the Glossary for abbreviations used in this article.

Introduction

Developmental cascades are generally headed by evolutionary conserved master regulators that determine the developmental fate of a cell lineage toward distinct tissues or organs during embryogenesis. In contrast, determination of the development of the reproductive organs does not follow this rule. Studies over the last decades have revealed that the gene-regulatory cascades triggering sexual differentiation from worms and flies to mammals are composed of substantially different factors. In particular, a remarkable diversity of master sex-determining genes that govern the genetic hierarchies has become apparent. On the other hand, the downstream components seemed to be evolutionarily more conserved and appear to converge on the regulation of a few central common effectors. A well-known example illustrating this paradigm is the master sex-determining gene of mammals, the SRY gene. A corresponding homolog has not been detected outside of therian mammals (Marsupials and Placentals). Conversely, those genes that act downstream of SRY as transcription factors (SOX9, DMRT1) or signaling pathways (TGF-β, Wnt4/β-catenin, Hedgehog), and genes involved in SRY regulation (SF1, WT1) have homologs with a known or presumed role in gonadogenesis or gonadal differentiation in many vertebrate species, and some even in non-vertebrate deuterostomes and protostomes. These findings suggested that a central paradigm of sex determination is that “masters change, slaves remain”.

This appealing global rule was quickly commonly accepted, in particular as the diversity at the top was confirmed experimentally [1–3]. Remarkably, some master sex-determining genes were recurrently identified and became the “usual suspects” for future studies in the search for master regulators (Table 1). All of these are genes, or duplicates and paralogs of genes, which were previously known to act in the regulatory network of gonad development. Much progress has also been made in understanding some of the regulatory interactions of the networks or cascades governed by the long known master sex-determining genes as well as, although to a lower extent, for the newly detected ones. We review here the current knowledge about the different molecules that have been demonstrated
Published online: September 9, 2015

Amaury Herpin & Manfred Schartl

Sex determination gene-regulatory networks

**Glossary**

**Amh**
Anti-Müllerian hormone

**Autosome**
On contrary to a sex chromosome, autosomal chromosomes are chromosomes that are not involved in primary sex determination

**Csd**
Complementary sex determiner

**CTD**
C-terminal domain

**Dkk1**
Dickkopf-related protein 1

**Dmd3**
Doublesex and Mab-3 domain family member 3

**Dmrt1 or 3**
Doublesex and Mab-3 related transcription factor 1 or 3

**Dosage sensitive gene**
Gene where the amount of gene product that determines the phenotype is dependent on the number of copies. Two copies are usually sufficient to establish the phenotype, while one is not (haplonsufficiency). For example, in birds two copies of the Dmrt1 gene trigger male gonadal development, while one copy is not sufficient to make a male and then leads to female development

**Dsx**
Doublesex

**Environmental sex determination (ESD)**
When the sex of an individual is driven by different external factors including temperature, pH, social interactions (dominance, stress...)

**Esr1**
Estrogen receptor 1 is the human estrogen receptor alpha

**Fem**
Feminizer

**FGF9**
Fibroblast growth factor 9

**Foxl2**
Forkhead box transcription factor L2

**Fru**
Fruitless

**Fst**
Follistatin

**Gene regulatory network**
Set of interactions between different regulators (DNA, RNA, proteins) leading to their interdependent modulation of expression and regulation

**Genotypic sex determination (GSD)**
When the sex of an individual is triggered by its genotype only (can be mono or polygenic)

**Gonadal maintenance**
Establishment of a genetic program in order to maintain the fate and differentiation state of the different cellular types composing the gonad, keeping either the male or female identity

**Gsdf**
Gonadal soma derived factor

**Her-1**
Hermaphrodization of XO-1

**Hetero-/homo- gamety**
When individuals produce gametes with either different sex chromosomes (hetero-) or similar sex chromosomes (homo-). It is referred to male heterogamety when males produce X and Y chromosome-containing gametes or female homogamety for females producing only X chromosome-containing gametes (XX-XY sex determination system, like in most mammals). For instance in birds, snakes and butterflies males are (ZZ) homogametic and females (ZW) heterogametic (ZZ-ZW sex determination system)

**Heteromorphic sex chromosomes**
When sexual chromosomes are morphologically distinguishable (different degrees of heteromorphism exist, depending on the age of the sex chromosomes)

**Hhip**
Hedgehog-interacting protein

**HMG**
High mobility group

**Irf9**
Interferon regulatory factor 9

**Mab-3**
Male abnormal 3

**Masc**
Masculinizer

**Master sex-determining gene**
A gene (not necessarily coding for a protein) responsible for the initial trigger leading to sex determination

**Neofunctionalization**
The process by which a gene changes its function or adds a new one by mutations that change the structure of its gene product and/or its expression pattern

**Nix**
Male-determining factor in the mosquito *Aedes aegypti*

**NTD**
N-terminal domain

**PiRNA**
PIWI-interacting RNA

**Primordial germ cells**
In the embryo the precursors of the stem cells that will give rise to the germ cell lineage. During sex determination and gonad differentiation they become committed to either produce male or female germ cells as spermatogonia or oogonia, which after meiosis will become the gametes. Primordial germ cells continuously express a certain set of genes in order to maintain their unique undifferentiated/pluripotent state

**Ptc1**
Patched

**Rspo1**
R-spondin 1

**Sdc**
Sex determination and dosage compensation defective

**SdY**
Sexual dimorphic on the Y chromosome

**Sex chromosome**
Chromosome involved in the primary sex determination. They usually harbour a master sex determining gene/trigger

**Sex determination**
Primary mechanism leading to the expression of the phenotypic sex. Sex determination is mostly triggered either by the genome (genotypic sex determination) or by the environment (environmental sex determination)

**Sexual differentiation**
Developmental consequence of the sex determination process. Regroups the events dealing with internal and external genitalia and secondary sex characters

**Sf31**
Steroidogenic factor 1

**Somatic gonad**
The non-germ line component of the gonad. The somatic gonad consists of mainly two characteristic cell types in female: the granulosa and theca cells of the ovary and three specific cell types in the testis: Sertoli, Leydig and peritubular myoid cells

**Sox9**
Sry-related HMG box 9
to determine sex in a variety of animals and what has been learned about the maintenance of the sexual identity of ovary and testis.

Master sex-determining genes: case studies from Sox and DM domain factors to emerging "unusual" suspects

From Sry down to Sox3 across vertebrates

SRY belongs to a family of transcription factors, which are characterized by an evolutionary conserved high-mobility group (HMG box) DNA-binding domain flanked by weakly conserved N- and C-terminal sequences. In mice, both, gain- and loss-of-function studies have shown that SRY is not only sufficient but also necessary for triggering testis development [4,5]. With the exception of only two species (the mole vole Ellobius [6] and the spiny rat [7]) which have probably lost the gene), SRY is the universal master male sex regulator of all therian mammals [8]. Cytogenetic and comparative molecular studies of mammalian sex chromosomes provided evidence that SRY most probably arose after two major events: (i) a dominant mutation of the SOX3 allele (giving rise to the proto-Y) as well as (ii) fusion of the gene with regulatory sequences from another gene already located on the X chromosome [9] (Fig 1). Necessarily occurring before the divergence of the therian lineage, these events could be estimated to have happened ~146–166 million years ago [10,11].

Sharing an overall identity of 67% at the amino acid level and up to 90% identity when specifically considering the HMG DNA-binding domain, the X-chromosomal SOX3-encoded protein is most similar to SRY [12]. Consistent with this hypothesis, the expression of SOX3 has been documented in the developing gonads of mice, chicken [13], fish [14], and frog [15]. Only the absence of SOX3 expression

Table 1. Master sex-determining genes in vertebrates.

<table>
<thead>
<tr>
<th>Master SD gene</th>
<th>Organism</th>
<th>SD system</th>
<th>SD gene ancestor</th>
<th>SD gene generated from ancestor by</th>
<th>Ancestor gene function</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRY</td>
<td>Therian mammals</td>
<td>XY</td>
<td>Sox3</td>
<td>Allelic diversification</td>
<td>Transcription factor, required in formation of the hypothalamo–pituitary axis, functions in neuronal differentiation, expressed in developing gonads</td>
</tr>
<tr>
<td>Dmrt1</td>
<td>Birds</td>
<td>WZ</td>
<td>Dmrt1</td>
<td>Allelic diversification</td>
<td>Transcription factor, key role in male sex determination and differentiation</td>
</tr>
<tr>
<td>DM-Y</td>
<td>Xenopus laevis</td>
<td>WZ</td>
<td>Dmrt1</td>
<td>Allelic diversification</td>
<td>Transcription factor, key role in male sex determination and differentiation</td>
</tr>
<tr>
<td>Dmrt1bY</td>
<td>Medaka (Oryzias latipes, O. curvinotus)</td>
<td>XY</td>
<td>Dmrt1</td>
<td>Allelic diversification</td>
<td>Transcription factor, key role in male sex determination and differentiation</td>
</tr>
<tr>
<td>SdY</td>
<td>Rainbow trout (Onchorhynchus mykiss)</td>
<td>XY</td>
<td>Irf9</td>
<td>Gene duplication</td>
<td>Interferon response factor, no gonadal function known</td>
</tr>
<tr>
<td>GsdfY</td>
<td>Luzon ricefish (Oryzias luzonensis)</td>
<td>XY</td>
<td>Gsdf</td>
<td>Allelic diversification</td>
<td>TGF-β factor, important role in fish gonad development</td>
</tr>
<tr>
<td>Sox3Y</td>
<td>Indian ricefish (Oryzias dancena)</td>
<td>XY</td>
<td>Sox3</td>
<td>Allelic diversification</td>
<td>Transcription factor, required in formation of the hypothalamo–pituitary axis, functions in neuronal differentiation, expressed in developing gonads</td>
</tr>
<tr>
<td>amhY</td>
<td>Perjerrey (Odontesthes hatcher)</td>
<td>XY</td>
<td>Amh</td>
<td>Gene duplication</td>
<td>Anti-Muellerian hormone, growth factor</td>
</tr>
<tr>
<td>amhr2Y</td>
<td>Fugu (Takifugu rubripes)</td>
<td>XY</td>
<td>Amh receptor 2</td>
<td>Allelic diversification</td>
<td>Type II receptor for Amh, important function in gonad development, medaka mutant shows sex reversal</td>
</tr>
<tr>
<td>Dmrt1</td>
<td>Chinese tongue sole (Cynoglossus semilaevis)</td>
<td>WZ</td>
<td>Dmrt1</td>
<td>Allelic diversification</td>
<td>Transcription factor, key role in male sex determination and differentiation</td>
</tr>
<tr>
<td>GsdfY</td>
<td>Sablefish (Anoplopoma fimbria)</td>
<td>XY</td>
<td>Gsdf</td>
<td>Allelic diversification</td>
<td>TGF-β factor, important role in fish gonad development</td>
</tr>
</tbody>
</table>
in the developing marsupial gonad is not consistent with a conserved role in mammalian sex determination [16,17]. Although SOX3 has no obvious primary function in sex determination, as the Sox3 knockout mice have no gonadal phenotype [18], the clear evolutionary relationship between SOX3 and SRY raised the question whether gain-of-function point mutations may account for SOX3-induced XX male sex reversal in mice or humans. This has been shown only recently using a transgenic mouse model in which ectopic expression of SOX3 in the developing XX gonads resulted in complete XX female to male sex reversal [19]. Interestingly, the XX gonads of the transgenic hemizygous mice (Tg/+X) did not only display an up-regulation of Sox9 but also started to differentiate Sertoli cells, forming testis cords together with the appearance of a male-specific vasculature. Interestingly, using co-transfection assays it was shown that, similar to SRY, SOX3 only modestly trans-activated the SOX9 testis-specific enhancer “TESCO” element [20] and synergistically interacted with steroidogenic factor-1 (SF1).

Interestingly, the development of SOX3-triggered testes in XX animals was not possible in the absence of Sox9. In the same direction, patients displaying XX female to male sex reversal due to rearrangements of the genomic regions encompassing the regulatory sequences of SOX3 have been reported [19]. Together, these data suggest that gain of function of SOX3 during gonadal development can in principle substitute for SRY to trigger testis development. These findings provide functional evidence supporting the long-standing hypothesis that SOX3 is the evolutionary precursor of SRY (Fig. 1). It is also reasonable to postulate that rearrangements of the SOX3 gene might be an underappreciated cause of XX female to male sex reversal in human patients [19].

While SRY appears to be specific to the therian mammals, there is accumulating evidence that SOX3 has spawned independently other sex chromosomes outside mammals. Though being expressed in the ovary of frogs [21] without any sex-determining function determined so far, sox3 might be involved in the switch responsible
for sex determination in the Japanese wrinkled frog (Rana rugosa). Members of this species are either ZW or XY depending on which side of the island they are located [22]. Curiously, the Z and X chromosomes are not only homologous but share many genes with the X chromosome of humans including the sox3 gene. Further molecular characterization and genetic mapping could disclose the presence of a Y-specific allele for sox3 [23,24]. So far, this is an intriguing finding, but further studies are needed to ascertain a function for sox3 in the sex developmental decision process of the embryonic gonad. If sox3 has such a function, then the next question would be how the different genetic systems (ZW or XY) impact on sox3 function.

Stronger evidence comes from the Indian ricefish (Oryzias dancena) (Fig 1), in which the XY sex chromosome pair also shares homology with the human X, including the presence of the sox3 gene [14]. Using positional cloning to identify the sex-determining locus, it was found that the male-specific region on the Y chromosome harbors a cis-regulatory DNA segment that up-regulates expression of the Y-chromosomal copy of sox3 during gonadal development (Fig 1). Sex reversal of XX fish transgenic for the regulatory segment linked to sox3 to become males, and fish with targeted deletion of the Y-chromosomal sox3 gene developing as females confirmed its major role during sex determination. Furthermore, it was demonstrated that sox3 initiated testicular differentiation by up-regulating expression of gsdf, a gene highly conserved in fish male sex differentiation pathways [14]. Interestingly, a BAC clone carrying the sox3 gene of O. dancena was not able to induce male gonadal development in the closely related species O. latipes, which has a different male sex determination gene. This suggests that the acquisition of sox3 function as a master sex-determining gene has occurred with a concomitant change in the downstream gonadal gene-regulatory network (Fig 1). Taken together, the results provided strong evidence for the identification of Sox3 into the pathway leading to male gonadal development.

SRY reveals plasticity of sex-determining mechanisms among mammals Despite substantial variations in expression profiles, structure, and amino acid sequences within mammals, the function of SRY to activate a conserved target gene—SOX9—during testis development appears to be conserved [20]. SRY directly binds to the TESCO sequence of the SOX9 gene [20]. Once activated, the SOX9 protein initiates the differentiation of somatic precursors into Sertoli cells that will then coordinate the gonadal development toward testes [25]. In the absence of SOX9 activation, the fetal gonad will develop toward ovaries. While the function of SRY as a regulator of SOX9 appears to be conserved, the molecular details underlying transcriptional regulation of SOX9 by SRY [26] are not fully known and their conservation among mammals has not been deeply investigated. Such information would be important to evaluate whether under a conserved master determiner, the subordinate network is strictly conserved as well or shows variation in its regulatory interactions.

In contrast to most known transcriptional activators, most SRY proteins that have been studied in different mammalian species do not exhibit a well-defined transactivation domain (TAD). For instance, the N- and C-terminal domains (NTD and CTD) flanking the evolutionarily conserved DNA-binding domain of human SRY are poorly preserved and do not seem to display any intrinsic transactivation activity [27]. Hence, it is assumed that the transcriptional activation of the human SOX9 gene by SRY is possible only after the recruitment of a transactivating protein partner through its NTD and/or CTD sequences [28]. However, mouse SRY does not only lack the NTD but also displays an unusual CTD made of a bridge domain together with a poly-glutamine (polyQ) tract encoded by a CAG-repeat microsatellite [27]. It has recently been shown that this poly-glutamine domain does not only prevent mouse SRY from proteasomal degradation, but additionally functions as a bona fide TAD. Due to the fact that it allows the direct transcriptional induction of Sox9, this poly-Q domain plays a central role for the male-determining function of SRY in vivo [27]. Such data suggest that during evolution, mouse SRY has gained a functional unit, which is absent in other mammals [27]. Given such important transactivating properties for that poly-Q CTD in mice, it is puzzling that SRY proteins from either human or goat lacking a TAD are able to induce testicular development in transgenic XX mice embryos [29,30]. It appears reasonable to consider that both human and goat SRY proteins are able to bind to the highly conserved mouse TESCO target sequence using their respective DNA-binding HMG boxes. For the activation of SOX9 transcription, it is assumed that transactivation is then mediated after the recruitment of a third TAD-containing protein partner. It can be further hypothesized that acquisition of a poly-glutamine stretch after insertion of a CAG microsatellite in a rodent ancestor made the recruitment of a transactivating partner unnecessary. Consequently, it is assumed that mouse SRY’s ability to employ such a transactivating partner was lost during evolution. This assumption is supported by the observation that the acquisition of the poly-glutamine stretch is concomitant with an increase of variation in different parts of the SRY protein. These include the loss of the NTD as well as accumulation of deleterious amino acid substitutions in the HMG box [31]. Though no longer required, the third partner protein—probably a pleiotropic effector—may still be expressed at the sex determination stage. It would then potentially enable human and goat SRYs to trigger male gonadal development when expressed in transgenic mice. This reveals an unanticipated level of plasticity of the molecular mechanisms in the implementation of the primary sex-determining signal even among mammals. Identification of such putative partners of SRY may help in understanding human primary sex reversal pathologies, which are not explained by alterations in the known players of male sexual development [32].

Roles of DM domain factors in sex determination, differentiation, and gonadal maintenance

DMRT1, wherever you look Among the evolutionarily conserved downstream effector genes of genetic sex-determining cascades, the DMRT gene family holds an outstanding position. This family is involved in sexual development of organisms as phylogenetically diverse as mammals, birds, fish, frogs, flies, worms, and corals [33–38] (Figs 2 and 3). Characterized by a highly conserved DNA-binding core motif—known as the DM (Doublesex and Mab-3) domain—, DMRT proteins act as transcription factors. Initially described to be involved in sex determination in worms and flies, they have been shown to regulate diverse aspects of somatic sexual dimorphism in these organisms. The ability to functionally
because of their recurrent subordinate role in the cascade. A deeper interest in the field of sex determination for this group of genes only came with the discovery of a dmrt1 homolog located on the Y chromosome of the medakafish (Oryzias latipes). Resulting from a gene duplication of the autosomal dmrt1a gene, it was designated dmrt1bY [41] or dmy [42]. It is the only functional gene in the Y-specific region of the sex chromosome, and it was shown to be not only necessary but also sufficient for triggering male development (see also Fig 2).

In humans, haploinsufficiency of the genomic region that includes DMRT1 and its paralogs DMRT2 and DMRT3 leads to XX male to female sex reversal [43]. This suggested that the DMRT1 gene is an important dosage-sensitive regulator of male development in vertebrates. In chicken and other avian species and in a fish, the smooth tongue sole (Cynoglossus semilaevis) [44]), DMRT1 is located on the Z chromosome, but absent from W, and shows the expected expression pattern for a dosage-dependent male sex-determining gene of birds [45] and flatfish. In chicken, it was demonstrated through RNA interference experiments that DMRT1 is indeed required for male gonad development [45]. While in these organisms DMRT1 acts as a dosage-dependent male determiner, in Xenopus laevis, a duplicated copy of dmrt1 on the W, which lacks the dimerization domain, appears to fulfill its function as a dominant-negative version. It is proposed to interfere with the transcriptional activation of the target genes of Dmrt1 and thus acts as a suppressor of male development [46].

Remarkably, all these DMRT1 genes have acquired their new roles as master sex determination genes through different mechanisms: via gene duplication and translocation in medaka, duplication, translocation and truncation in Xenopus, or loss of function of the W allele in birds or tongue sole (Table 1).

In mice, it is apparent that Dmrt1 is not required for male primary sex determination since newborn Dmrt1 mutants are males with testes [36]. However, Dmrt1 is required for male gonadal differentiation of somatic cells and germ cells [47–49]. This is a parallel situation to mammalian Foxl2 [50], which plays a conserved role in ovarian development but in mouse (opposed to some other mammals, including human and goat [51]) is not required for initiation of female development (see [52] for review). Targeted deletion of mouse Dmrt1 and also of the autosomal dmrt1a of medaka, which is not involved in primary male sex determination, have revealed a major role in male gonad maintenance: when Dmrt1 is lost, even in adults, this triggers sexual cell-fate reprogramming, in which male Sertoli cells trans-differentiate into their female counterparts, the granulosa cells [49]. This is accompanied by testicular reorganization toward a more ovarian morphology [49]. Ectopic DMRT1 expression in the ovary silenced the female sex-maintenance gene Foxl2 and reprogrammed juvenile and adult granulosa cells into Sertoli-like cells, triggering formation of structures, which resemble male seminiferous tubules [53]. In the same direction, deletion of the dmrt1 gene in medaka resulted in transition of the developing testis to ovary [54]. Hence, DMRT1’s range of action is not limited to function in initiating the male gonadal phenotype during early development but also accounts for the livelong active repression of the two “anti-testis” pathways of FOXL2 and WNT4/β-catenin [49], and can do so even in the absence of the testis-determining genes SOX8 and SOX9 (Fig 2). Additionally, mRNA profiling revealed that DMRT1 activates many testicular genes and

---

**Figure 2. Gene-regulatory network of gonadal sex induction and maintenance in vertebrates.**

Schematic representation of main interactions within the regulatory network. In gonadal fate determination of mammals, Sry initiates activation of the male pathway (blue) through up-regulation of Sox3. Dmrt1 is not only important for keeping the male pathway on, but also in suppressing the two female networks (red). These two female networks involve Foxl2 as well as the Wnt/β-catenin signaling pathways. Maintenance of gonadal identity in the differentiated gonads is a result of the cross-inhibition activities of Dmrt1 and Foxl2. A critical equilibrium between these conflicting pathways underlies the biopolarity of the gonadal somatic cells. Tipping the balance into one direction or the other will regulate the gonadal fate as a consequence of the activation of the male or female pathways. Solid lines define negative regulations. Dashed lines designate positive regulations. Beside the Sry ancestor Sox3 and Dmrt2, other genes (pink) can become the master sex-determining genes by similarly impacting on the seesaw between the male and female programme.
down-regulates ovarian genes [53]. Interestingly, transient expression of DMRT1 has also been reported in the fetal gonad of both sexes. The involvement in the regulation of germ cell development in testes and oocytes indicates that DMRT1 has different functions in males and females [55].

DMRT1 is required in female germ cells for entry into meiotic prophase, and in male germ cells for the control of mitotic arrest until birth [55]. Control of the decision to enter meiosis versus mitotic arrest is mediated by the ability of DMRT1 to selectively modulate retinoic acid signaling through context-dependent regulation of STRA8. DMRT1, for example, directly represses STRA8 transcription during testicular differentiation [55]. Thus, a picture emerges where DMRT1 controls a regulatory network that on the one hand can drive sexual fate and on the other hand can maintain the program of sexually differentiated cells, depending on the cellular context.

DMRT1, a jack-of-all-trade From studies in mouse and medaka [49,53,54,56,57], it is emerging that DMRT1 holds a key position as the master switch or gatekeeper controlling the cell fate of the somatic cells of the gonads in female and male [33,34,53,58,59]. If this is so, then one could ask, why such a complex regulatory network upstream of DMRT1 would be necessary to flip the switch, because numerous examples indicate that DMRT1 can do it on its own as for instance in birds, Xenopus and medaka [41,42,45,46]. DMRT1 orthologs in these species appear to have undergone mutational events causing either loss or gain of function. Such altered DMRT1 activity may have favored evolutionary transitions leading to new genetic sex determination systems (see [59] for review). The ability of DMRT1 to toggle Sertoli/granulosa cell fate supports the hypothesis that loss- or gain-of-function mutations in DMRT1 can elevate it into a master sex-determining role. Such mutations would help to promote changes between genetic sex determination mechanisms that are commonly observed among vertebrates.

DMRT1 is one of the sex determination network genes that appears more often also as master regulator (Table 1). It can be hypothesized that its strategic position at the interface of sex determination and the process of sex-specific gonadal differentiation, integrating a developmental fate decision with activation of organ differentiation programmes (Fig 2), made DMRT1 suitable to be selected either as new controller at the top or at least for being one of the few key genes to be regulated.

Emerging suspects from gonadal TGF-β signaling The anti-Müllerian hormone (Amh) is a growth factor from the TGF-β family and plays a major role in mammals for the degradation of the Müllerian duct-forming part of the female reproductive tract in male embryos. It is not required for mouse testis development. However, in non-mammalian vertebrates, it appears to play a central role in testis formation. For instance, in chicken embryonic gonads, AMH is expressed much higher in males and is predicted to be responsible for organizing the early testis in birds [60]. In the medaka hotei mutant, Amh signaling is disrupted by a mutation in the type II receptor for Amh. As a consequence, a male to female sex reversal with an over-proliferation of germline stem cells occurs [61]. Although being clearly a subordinate member of the sex regulatory network in mammals and at least in those species that make use of DMRT1 as master regulator of male development, the Amh/Ahm-receptor system has, like DMRT1, sometimes made it to the top (Table 1). In the pejerrey, a freshwater fish species from Patagonia, a duplicated version of the amh gene became the male sex-determining gene on the Y chromosome [62], reminiscent of the situation for dmrt1 in medaka fish. In the pufferfish, Fugu rubripes, the receptor for Amh exists in two versions that differ by one amino acid (H384D) in the kinase domain [63]. The 384His allele is a Fugu-specific (conserved in several other pufferfishes) mutation that confers lower activity to the receptor and is encoded on the X chromosome [63]. Thus, a quantitative difference in Amh signal transduction in females, which are homozygous for the mutant, versus males, which have kept one allele of the wild-type receptor on their Y, is responsible for male development [63]. Like in the medaka hotei mutant [61], low signaling from the receptor is connected to feminization of the gonad.

Gonadal soma-derived factor (Gsdf) is another growth factor from the TGF-β family that is closely related to Amh. It is only found in fish, and its biochemical function is not well studied. It is assumed to have a role in male gonad development due to its exclusive expression in the early differentiating testis of all fish looked at so far [64–68]. Despite its proposed role in the downstream regulatory network, gsdf has made it up to the top in Oryzias luzonensis [69] a sister species to medaka, and most likely also in the sablefish [70].

Taken together, it appears that certain genes, which are members of the regulatory network, namely sox3, dmrt1, and TGF-β signaling components, can become the master sex-determining gene independently again and again, while other important components of the sex-determining pathways have not appeared as masters so far (Fig 2 and Table 1). Whether we just have to wait for the analyses of primary genes for sexual development in more species, in order to put genes like foxl2, sox9, sox8, wnt4, etc., on the list of usual suspects, or whether there is a biological reason that makes some genes more prone to become the top regulator, is currently unsolved. We could imagine that some genes remain “too difficult to recruit” as master regulators, for instance if they have also non-reproductive but vital functions in other organs. In such case, interferences between a duplicated new master gene and its homolog may not be tolerated, except for the case that the neo-gene would have an appropriate gonad-specific regulation as soon as the founder event occurs. Many of those genes that did not appear as master sex determiners so far indeed have important functions in other tissues and organs.

Recurrent actors in invertebrate sex determination The invertebrate ancestors of DMRT1 DM domain-containing genes have been shown to be primarily involved in gonad differentiation in a flatworm [39] and to direct male versus female development of dimorphic structures in water flea [40]. Interestingly, this functional convergence is common among insects (see [3,71–73] for reviews). In Drosophila, the initial trigger of sex is dependent on the ratio of the number of X chromosomes versus the haploid autosome complement (X:A). In the female situation, an X:A ratio of one will enable the transcription of the Sex lethal gene (Sxl), a splicing regulator. The SXL protein will then promote the female-specific splicing of Transformer (Tra), a direct downstream target, and lead to the production of functional TRA proteins. Similarly, a complex made of TRA and TRA-2 proteins will then favor the female-specific
splicing of the *Doublesex* (*Dsx*, the *Dmrt1* homolog) gene transcripts. This results in the production of the female-type DSX protein DSX^F^, which initiates up-regulation of the downstream gene regulatory network for female development. In males, an X:A ratio of 0.5 will prevent the production of the SXL protein and, by default, results in the production of the male-specific splice form of the *Dsx* gene. This splice variant translates into a non-functional protein due to a premature stop codon. In the absence of TRA, by default the male-specific splice form from the *Dsx* gene will be produced. The male-type DSX protein DSX^M^ will then orchestrate the downstream splicing events in the molecular regulation of sex determination and so far, it is not reported that a DM domain

Despite considerable efforts, similar sex-specific alternative splicing events in the molecular regulation of sex determination of vertebrates have not been shown. Conceptually similar is the fact that DSX translates the sexual determination process of a cascade of alternative splicing events into the transcriptional control of a large number of sex-specific effector genes. Similarly, DMRT1 in vertebrates appears to hold such a “translational” function at the interface where a fate-determining signal is put into effect at the level of sex-specific somatic cell differentiation (Figs 2 and 3).

In invertebrates, the homologs of vertebrate *Dmrt1* (e.g. *Dsx* in *Drosophila* and *Mab3* in *C. elegans*) are typical downstream factors of sex determination and so far, it is not reported that a DM domain
gene has made it up to the top in any invertebrate species [3]. But like in vertebrates, genes that are known as downstream members in one species can also usurpate a position as an initial genetic trigger in another species [3]. In insects, paralogs of the gene \( tra \) that is a well-studied component of the sex determination cascade in \( Drosophila \), evolved as the master sex-determining switch gene in the housefly (\( Musca domestica \)), a wasp (\( Nasonia vitripennis \)), and the honeybee (\( Apis mellifera \)) [72,79,80]. In this regard, studies about complementary sex determination in the honeybee give exciting insights into how molecular diversity of regulatory pathways can evolve [81,82], as discussed in more detail below.

### Complementary sex determination in honeybees uses a conserved module from chromosomal sex determination

Genetic sex determination in the honeybee does not depend on the presence of hetero- or homomorphic sex chromosomes with different genetic compositions but rather follows a haplodiploid mode. Males develop from haploid unfertilized eggs, while diploid fertilized eggs develop into females. Hence, male or female sexual development occurs as the result of a signal originating from either a single or two different alleles from one gene, called complementary sex determiner (Csd) (Fig 3). Consequently, maleness or femaleness is determined by either homo-, hemi-, or heterozygosity of the Csd locus. The Csd gene products belong to an arginine-/serine-rich protein family. Interestingly, the C-terminal end of Csd also displays high similarity with the TRA protein, an essential downstream genetic factor of the sex-determining pathway in \( Drosophila \) ([81] and Fig 3).

Intriguingly and in contrast to the situation in \( Drosophila \) with \( Tra \) and other downstream genes (see Fig 3), neither transcriptional nor splicing variations of the Csd gene could be detected as sex-specific triggers. It is currently presumed that the regulation of the downstream regulatory network is mediated by the tendency of the CSD proteins to form heterodimers. Interestingly, the sex determination locus of the honeybee harbors a second gene also required for sex determination: feminizer (Fem) [82]. Further, phylogenetic studies revealed that Fem— as Csd—is also a close homolog of the \( Tra \) gene from \( Drosophila \). It has been shown that \( Csd \) arose after duplication of the Fem gene. Fem is expressed during gonadal differentiation, was recently characterized [85]. Localized at the sex-determining locus, this gene was named \( sdY \) for sexual dimorphism on the \( Y \) chromosome. Astonishingly and unlike other master sex-determining genes characterized so far, \( sdY \) has no homology with any known gene in sex determination pathways but with an immunity-related gene, the interferon regulatory factor \( irf9 \) [85]. \( sdY \) arose by duplication and truncation of the autosomal \( irf9 \) gene (Table 1). It lost the DNA-binding domain but preserved its protein–protein interaction domain. So far, the molecular mechanism through which \( sdY \) triggers male gonad development is unknown.

### An immune-related gene evolved into the master sex-determining gene in rainbow trout

In the rainbow trout \( Oncorhynchus mykiss \), a gene expressed only in the testis, predominantly during testicular differentiation, was recently characterized [85]. Localized at the sex-determining locus, this gene was named \( SdY \) for sexual dimorphism on the \( Y \) chromosome. Astonishingly and unlike other master sex-determining genes characterized so far, \( SdY \) has no homology with any known gene in sex determination pathways but with an immunity-related gene, the interferon regulatory factor \( irf9 \) [85]. \( SdY \) arose by duplication and truncation of the autosomal \( irf9 \) gene (Table 1). It lost the DNA-binding domain but preserved its protein–protein interaction domain. So far, the molecular mechanism through which \( SdY \) triggers male gonad development is unknown.

### A single female-specific piRNA is the primary determiner of sex in the silkworm

Sex in the silkworm \( Bombyx mori \) and all butterflies is determined by a \( ZW \) sex chromosome system. The \( W \) chromosome lacks any protein-coding genes but consists predominantly of transposons and non-coding RNAs. The only transcripts produced from the sex-determining region on the \( W \) are PIWI-interacting RNAs (piRNAs). After deep sequencing and isolation of dimorphically expressed RNAs, the Fem piRNA (Fem standing for “feminizing factor”) was shown to be specifically expressed in females at all stages of development [86]. Furthermore, Fem piRNA targets and cleaves the Masculinizer (Masc) RNA molecule transcribed from a gene located on the \( Z \) chromosome. Interestingly, Masc, a CCCH-type zinc finger protein, favors male-specific splicing of \( Bm-dsx \), leading to male development [86]. Hence, in \( ZW \) embryos, Masc RNA level is down-regulated by Fem piRNAs, inhibiting male development. By default, female-specific splicing of \( Bm-dsx \) then occurs, triggering female development [86] (Fig 3). Interestingly, genetic inhibition of Masc resulted in the premature death of ZZ embryos before they hatched. In light of this observation, it was shown that the Masc protein is necessary for dosage compensation in order to lower \( Z \) gene transcription in ZZ embryos to the same level as in \( ZW \) embryos [86]. Whether or not this sex determination pathway is conserved across all lepidopterans remains to be explored, but coupling the most important mechanisms namely sex determination and dosage compensation within the same genetic pathway and additionally distributing their genes onto the sex chromosomes should strongly promote evolutionary conservation.

\( SdY \) from rainbow trout and Fem piRNA are paradigms showing that unrelated genes are able to acquire \textit{de novo} sex-determining functions. It can, however, not be excluded that they are representing...
factors of the sex determination regulatory network that have been overlooked so far.

**Plasticity of the downstream sex determination regulatory network**

What happens when “masters change”? The slogan “slaves remain” could imply that not much happens downstream of the changing master sex determinant. However, the findings on the diversity of SRY structure and its way to act as a transcriptional activator (see above) indicate that even under the same master gene, the regulatory interactions of the network undergo changes and that biology is not that simple.

In *Drosophila*, it has been shown that at the very downstream end of the sex determination, cascade pathways diverge by cooption of new effector genes [73] explaining the divergence of secondary sex characters between species. In vertebrates, some transcription factors like DMRT1, FOXL2, SOX9, and components of pathways such as Rspo1/Wnt/Fst or Hedgehog of the gonadal gene-regulatory network are well conserved on the DNA sequence level; however, their specific functions, regulations, and interplays can be substantially different. In medaka, down-regulation of the Hedgehog pathway by Dmrt1bY was shown [87]: Transcription of the Hedgehog receptor Ptch-2 in medaka testis is down-regulated by Dmrt1bY in the medaka testis, together with an up-regulation of *Fst* and *SOX9* [90], the Hedgehog pathway might not only be dispensable during medaka male gonadogenesis and maintenance, but needs to be suppressed by *DMRT1* genes.

For R-spondin 1 (*Rspo1*), preferential ovarian expression is generally described. However, such strict female dimorphism was not observed in zebrafish [91], where the gene is also expressed in adult testes. Here, Rspo1 has a crucial role in testis cell proliferation [92] and it has further been shown to be involved in skin and mammary gland differentiation in mammals [93]. Follistatin (*Fst*) expression in the mouse co-localizes with *Foxl2* in the ovary [94], but in rat, it is expressed very broadly in germ and somatic cells of the testis [95]. Sparse expression of *fst* was also noted in the interstitial cells of the medaka testis, together with an up-regulation of *fst* expression in *vivo* after transfection of *dmrt1a* [87].

**SOX9** has been shown to be expressed in the developing testes of all vertebrate embryos examined so far (see [60] for review). However, whereas SOX9 is upstream of AMH in mammals, the reverse applies in birds, and in medaka, Sox9 even appears to be not involved in primary sex determination at all [96,97]. In mammals, the current understanding is that SRY acts together with SF1 to activate SOX9, while in return, SRY is turned off by SOX9. SOX9 further maintains its expression in an autoregulatory loop. SF1 is still required, but SRY becomes dispensable later during development [20]. In non-mammalian vertebrates, Sox9 activation must then rely on other factors than Sry. Intuitively, one could think that DM domain genes might have taken over. However, in chicken embryos, DMRT1 expression is occurring at least 2 days before that of SOX9 [60], implying that other genes mediating the DMRT1 signal to SOX9 are involved. In medaka *sox9b*, the homolog of tetrapod *sox9* genes is rather involved in germ cell function than gonad determination although being expressed in the somatic part of the primordial gonads [96]. In addition, while in mammals, SOX9 activates the expression of FGF9 [98], the gene does not exhibit any sexually dimorphic expression in chicken [60] and has even been lost in fish [99]. It is obvious that the gonadal function of SOX9 underwent several changes during vertebrate evolution.

Genetic networks are indeed more complex than a straight top-down scenario. We have to add now that the differences in gene expression do not only reflect differences in cell biology and morphogenesis of the gonads but definitively are also the consequences of changes in the initial trigger for activating the network. That master sex-determining genes are prone to regulatory putsches in order to acquire an upstream position might only be possible because of the flexibility of the downstream gene-regulatory network. Hence, while Graham proposed a few years back that “Masters change, slaves remain” [1], it is now time to change this paradigm: “When masters change, some slaves remain, others are dismissed or acquire new tasks, and new ones can be hired”.

**Conclusions and perspectives**

The variability and plasticity of the mechanisms that govern the development of the gonads is unmet by any other organ systems or tissues. While for instance the Pax6 gene that is a master regulator of mammalian eye development is highly conserved (ectopic expression of human *PAX6* is able to induce eye development in *Drosophila* [100]), the downstream components of this cascade are not conserved (the induced eye is a typical composite insect eye). Surprisingly, it appears to be the other way round for sex determination genes. The evolution of genetic interactions in the sex-determining pathways and cascades is characterized by a relative conservatism at the bottom and an apparent diversity at the top. This was explained in a classical hypothesis by A. Wilkins with an evolutionary scenario in which these hierarchies during evolution build up from a common downstream component (Sox or DM domain factors for instance), which acquires new upstream regulators. Those new additions would naturally vary in different evolutionary lineages [101]. Recent studies on the molecular identification of such upstream regulators and the downstream regulatory network, some of which provided the backbone for this review, brought new insights into how sexual development is regulated in different organisms, and how new sex determiners have evolved.

The “bottom-up hypothesis” formulated by Wilkins has to be revisited now taken into account the discoveries of the new master regulators. It seems that the master regulator switch is not necessarily elected from the existing cascade usurping the top position but could be equally recruited from outside to accomplish a new sex-determining function after neo-functionalization. We also have to modify the hypothesis as we now know that in vertebrates, unlike in invertebrates, sex determination is not brought about by a simple linear cascade, but by a complex network of multiple regulatory interactions. Such a network might offer multiple opportunities where a newly added factor can trigger the outcome of the network signal toward male or female. There is also evidence accumulating
that regulatory cascades can become shorter, rather than being topped up, when a new sex determiner appears, for example, in honeybees [72,102].

Gonad development appears to cope well with such changes of primary triggers as the many examples of different master sex regulators show, which finally all guarantee the developmental switch to either a testis or ovary. An intriguing situation has been recently reported for zebrafish, where the laboratory strains used worldwide have all lost their original sex-determining chromosome, but still produce normal males or females [103]. New upstream sex determiners appear to evolve quickly in those domesticated strains—similar to a situation in the other small aquarium fish model, the medaka [104]—which might take care in the future of the current sex bias observed at present for many laboratory strains. These are instances of “evolution in action,” which offer prospects to observe in the laboratory how new sex determiners evolve and to obtain insights into the underlying molecular mechanisms. Certainly, we also need more information from different species about their master sex-determining gene and how it acts on the downstream regulatory network to obtain a reasonable understanding of the variety of sex-determining mechanisms.

Somewhat unexpected are the accumulating findings that also the downstream network is not as strictly conserved as the “masters change, slaves remain” paradigm was imposing. Whether these differences in the expression pattern and function are related to specific adaptations of varying reproductive biology is a challenging question for the future. On the other hand, such changes may be due to the impact of the new upstream regulator. Intriguingly, even in a setting of the same master sex-determining genes, intricate differences downstream can be found, as seen for SRY in different mammals. It has also been argued that genetic networks, including sex determination, in general can change randomly without necessarily impacting on the final phenotype and thus evolve neutrally (see Sidebar A). Again, we need more details on the molecular biology of the sex-determining networks from different organisms; for instance, on a comparative basis from birds, Xenopus and those fish that all use dmrt1 as their common master sex-determining gene.

Unexpectedly, it turned out that sex determination is not only needed as the molecular switch for the undifferentiated gonad primordium to develop either as testis or ovary, but that the sexual identity of the gonadal soma needs to be maintained as long as the organ has to provide its function(s). In vertebrates, two genes that appear to have a more downstream function in the determination network of the embryo are the top players here: DMRT1 and FOXL2. The dichotomous developmental potency of the gonadal soma is apparently kept throughout the entire life. The reason for this is unknown. In particular among fishes, hermaphroditic species are common. Those fish can switch during their reproductive life from one sex to the other. Whether these organisms have found a way to make a controlled use of the livelong plasticity of the gonad or whether the plasticity seen even in the mammalian gonads is a relic of an evolutionary past are just two questions that emerge from those new findings.

The recent progresses reviewed here have considerably increased our understanding of the diverse molecular mechanisms underlying the amazing variation and plasticity of sexual development, and we might so far just only see the tip of the iceberg.

Sidebar A: Evolutionary concepts for the diversity of sex determination mechanisms

Sex determination is a very basal and ubiquitous developmental process, and the fact that it is so variable even between closely related organisms poses many fascinating questions. Molecular biologists are most interested to understand how these different mechanisms work, what factors are involved, upstream and downstream, and how they are regulated to bring about the amazing plasticity of the respective genetic cascades and networks. These are the so-called proximate causes of the observed variability. Organismic biologists focus more on the “ultimate” causes that lead to the changes from one to the other sex determination mechanism within and between certain lineages. A number of scenarios and hypotheses have been put forward to explain which evolutionary forces could favor such transitions and turnovers [105].

One explanation is that a mutation, which creates a new sex determination mechanism, gives a fitness advantage to its carriers. Then, by natural selection, this mutation will sweep through the population and take over, while the previous mechanism is lost [106]. Such new mutations could for instance alter the sex ratio, and if the ecological conditions favor such a bias, this mutation will be beneficial. As another example, a new sex determination mechanism might for instance be more efficient under certain ecological conditions, for example, works faster or is less or more susceptible to environmental influences. If sex is determined through sex chromosomes, a common feature is the reduction of recombination around the sex-determining gene, which spreads out from there over almost the entire chromosome and finally fully arrests. As a consequence, deleterious loss-of-function mutations will accumulate in genes on the chromosomes carrying the sex locus [107]. Hence, such a chromosome will become less fit in evolutionary terms because of its mutational load, and once these disadvantages accumulate to a critical level, an emerging “younger” and less degenerated sex chromosome can take over [108].

Another hypothesis is based on linkage of sex-determining genes to other genes that favor one sex or are antagonistic to the other sex [109]. Many examples exist for such genes, which for instance are involved in gonad development or sexual dimorphism. If such a gene is closely linked to a gene that can influence the developmental decision toward male or female, the sex-determining gene will be co-selected as a hitchhiker and enjoy the fitness advantage that the linked sex beneficial or sexually antagonistic gene has under conditions of natural or sexual selection. Rather than postulating a fitness advantage for the emerging novel sex determination mechanism, it is also considered that neutral or non-adaptive processes of genetic drift, mutation, and recombination can be instrumental. Such hypotheses are based on an analysis by M. Lynch how in general genetic networks can evolve [110]. He pointed out that only the final gene product of a genetic network or cascade produces a phenotype, which is exposed to selection. Thus, many changes in the upstream system can occur without necessarily altering the finally expressed phenotype. These changes can become fixed in a population by random genetic drift. As a result, the regulatory network has changed, but the phenotype will be constant. Such considerations were then applied to the genetic cascades and networks that govern sex determination [102]. Indeed, the final outcomes of the sex determination process are morphologically and functionally surprisingly similar in related groups of organisms, which have very different master sex regulators [111].

For all of these theoretical explanations, which appear to be to a certain extent opposing or even contradictory, examples to support them can be found. A single one obviously cannot explain all the different cases of sex determination systems and the multitude of turnovers and transitions. Rather than being alternatives, they may be complementary to explain the biodiversity of mechanisms that make the undifferentiated gonad anlage of an embryo to develop toward testis or ovary. To further our understanding of the trajectories that lead to the evolution of diverse mechanisms, we need not only detailed molecular knowledge about the proximate causes of such diversity but also more information about the ecology and population genetics under which they occur.
Sidebar B: In need of answers

(i) What are the protein partners of SRY in human and goat that directly activate Sox9 expression?

(ii) Are the differences in the expression pattern and function of the genes in the downstream cascades or networks related to specific adaptations of varying reproductive biology? Or are they the result of neutral evolution and genetic drift?

(iii) Have the naturally occurring hermaphroditic species of fish found a way to make a controlled use of the livelong plasticity of the gonad? Or is the plasticity seen in the mammalian gonads a relic of an evolutionary past?

(iv) What are the evolutionary forces driving the outstanding high variability of molecular and genetic mechanisms of sex determination? Is this all due to stochastic variation? Or is there a global (so far unknown?) reason? Or do all evolutionary mechanisms postulated so far cooperate, with differing importance depending on the species or phylogenetic lineage?

(v) Are Sox9 and Irf9 in vertebrates and Fem piRNA components of the downstream sex determination cascades or networks that have been overlooked so far?

(vi) Why do some members of the regulatory networks of sexual development frequently become master sex-determining genes while others never appear at the top position?

Acknowledgements

We thank Yann Cuiguen (INRA, LPCP, Rennes), Matzeu Adolfi, Sylvain Berthon, and Alvaro Roco (Biocenter Würzburg) for help in preparing the manuscript, and Monika Niklaus-Ruiz for help in preparing the manuscript. Work of the authors was supported by the Deutsche Forschungsgemeinschaft (Scha408/12-1, 10-1; He7135/2-1) and the ANR (ANR-13-ISV7-0005 PHYLOSEX; Crédits Incitatifs Phase 2015/1Emergence to A.H.).

Conflict of interest

The authors declare that they have no conflict of interest.

References


Sex determination gene-regulatory networks

Amaury Herpin & Manfred Schartl

The Authors


Sex Determination

- History
- Jost model of sexual differentiation
  - Chromosomal sex
  - Gonadal sex
  - Phenotypic sex
- Gonadal development systems
  - Required genes
  - How does chromosomal sex dictate gonadal sex?
    - Molecular cloning of testis-determining factor(s) (e.g. SRY)
    - Interactions of SRY and SOX genes
    - X chromosomes sex determining factor DAX/DOAX
    - Interactions DAX, DSS, SOX, and DMRT
- How does gonadal sex dictate phenotypic sex?
  - Müllerian inhibitory substance (MIS)
  - Androgen induced male differentiation
- Abnormal sexual differentiation
  - New potential sex determination genes
  - Mechanisms of sex determination in other species

Discussion Outline (Sex Determination)

Primary Papers:

Required Reading


History and Jost

![Diagram of sex determination process](image-url)
Jost Model -
Alfred Jost, University of Paris
1940’s & 1950’s
The human and chimpanzee Y chromosomes differ considerably in organization and structure despite the recent divergence of the two lineages (about 6 million years ago). Ampliconic regions contain massive palindromic arrays of multi-copy testis-specific genes. X-degenerate regions denote segments homologous to the X chromosomes that were present on the proto-sex chromosomes before they started to diverge into X and Y. The X-transposed region on the human Y chromosome is a segment transposed from the X chromosome to the Y chromosome in the human lineage after the split from chimpanzee. Most parts of the human and chimpanzee Y chromosomes do not recombine during male meiosis and are referred to as the male-specific region on Y (MSY). The figure is modified, with permission, from Ref. 438.

Sequence classes, genes, and palindromes on the human Y chromosome. (a) Schematic representation of the entire human Y chromosome, with the male-specific region (MSY) indicated. (b) A more detailed representation that focuses on the euchromatic MSY and excludes the major heterochromatic block on Yq. Palindrome arms are shown as pairs of dark blue triangles. Arms belonging to the same palindrome are indicated by adjacent, opposite-facing triangles. Positions of protein-coding genes are indicated directly below the chromosome by vertical lines. The TSPY gene family, indicated by a rectangle below the chromosome, is arranged in a tandem array with 23–64 copies of a 20.4-kb repeat unit. Each repeat unit contains a single copy of the TSPY gene. (c) Locations and sizes of recurrent deletions within the human MSY that cause (or, in the case of the gr/gr deletion, predispose to) spermatogenic failure. Abbreviations: PAR1, short-arm pseudoautosomal region; PAR2, long-arm pseudoautosomal region.

Mechanisms by which b2/b4 and gr/gr deletions arise. (a) The organization of amplicons (large, nearly identical segmental duplications) that make up the AZFc region. Amplicons of the same color have DNA sequences that are 99.82%–99.97% identical to one another. b2/b4 deletions are caused by ectopic recombination and crossing over between the b2 and b4 repeats in the AZFc region. (b) Schematic representation of the b2/b4 crossover, which could occur either within a single chromatid or between sister chromatids. (c) The product of the b2/b4 crossover, an extremely abbreviated variant of the AZFc region from which 3.5 Mb have been removed. (d) Ectopic crossing over between green or red amplicons, causing gr/gr deletions. Abbreviations: cen, direction of centromere; q ter, direction of long-arm terminus of the chromosome.
Gonadal Sex Determination
Fig. 1. Diagrammatic representation of parthenogenetic development in mammals. The development of the genital ridge into a testis or an ovary proceeds via a series of morphological stages. These stages are characterized by the differentiation of the genital ridge into ovarian or testicular tissue. A series of specific stages leads to the formation of the reproductive organs.
The establishment of the bipotential genital ridges and gonadal sex determination. In mammals, the genital ridges (blue) typically appear as longitudinal outgrowths along the surfaces of the mesonephros within the coelomic cavity. In mice, they emerge at ∼10 dpc through recruitment of cells from the overlying coelomic epithelium (brown). Primordial germ cells (yellow) colonize the genital ridges (arrows) after leaving the hindgut (red) via the dorsal mesentery. At this stage in development, the genital ridges are bipotential and can differentiate into testes or ovaries, depending on genetic cues. From ∼10.5 dpc, the Y-linked sex-determining gene Sry is expressed in XY genital ridges and initiates Sox9 expression and testis differentiation. In the absence of Sry, as in XX genital ridges, ovary differentiation is initiated by the action of genes such as Rspo1 and Wnt4.

Figure 2 | Compartmentalization of the testis. a | At the earliest stages of testis organogenesis (11.75–12.0 days post coitum; dpc), Sertoli cells (stained with SF1 antibody; blue) polarize and begin to aggregate around clusters of primordial germ cells (stained with PECAM antibody; asterisk) to initiate development of testis cords. A basal lamina is deposited between Sertoli cells and peritubular myoid cells (PM). The interstitial compartment contains Leydig cells (L; yellow) and the coelomic vessel (CV; red), with branches that extend between cords.
Known and proposed origins of the testicular cell lineages. The cells of the nascent genital ridges originate primarily from the overlying coelomic epithelium but also from the subjacent mesonephros. A subset of ingressing coelomic epithelial cells differentiates into Sertoli cells following Sry expression. Some of these supporting cells are also believed to differentiate into FLCs. It is unclear whether cells originating from the mesonephros contribute toward somatic cells other than blood endothelium, but they very likely contribute to the mesenchyme. The origin of PMCs remains unknown, but it is likely that they differentiate from a subset of mesenchymal cells or yet unidentified precursor cells of the testis interstitium. A second origin for the FLCs has also been proposed to include perivascular cells located at the gonad–mesonephric junction.

Figure 4 | Cellular events downstream of Sry rapidly organize testis structure. At the bipotential stage (10.5–11.5 days post coitum; dpc), no obvious morphological features distinguish XX and XY gonads. Antibodies against laminin (green) outline all cells in the gonad (G) and also label the basal lamina of mesonephric tubules (MT) in XX and XY samples. In XY gonads, Sry upregulates nuclear SOX9 (blue) in pre-Sertoli cells, and initiates Sertoli-cell differentiation by 11.5 dpc (germ cells and vasculature are labelled with platelet endothelial cell adhesion molecule (PECAM); green). Between 11.5–12.5 dpc, male-specific pathways activate marked morphological and cellular changes in the XY gonad (left column) that do not occur in the XX gonad (right column). These include an upregulation of proliferation in coelomic epithelial cells (measured by BrdU incorporation; red, arrow); migration of cells from the mesonephros (detected in recombinant cultures between a wild-type gonad and a mesonephros in which all cells express GFP; green); structural organization of testis cords (detected by laminin deposition; green); male-specific vascularization (red; blood cells are visible in the light microscope; arrow); and Leydig-cell differentiation (detected by RNA in situ hybridization for the steroid enzyme, Scc). BrdU image pair reproduced with permission from Ref. 29 © (2000) The Company of Biologists Ltd. XY migration image and vascular image pairs reproduced with permission from Ref. 77 © (2002) Elsevier Science.
**GENES REQUIRED TO OBTAIN BIPOTENTIAL GONAD**

- Found with knockout mice or mutant human tissues not having gonad form from genital ridge.

**WT1** - Wilms’ Tumor, WAGR Syndrome, Fraser Syndrome, Derys-Drioli Syndrome
- Sex reversal/different pathologies
- Four zinc finger domains
- 16 different products from gene, 11 p13

**SF1** - Steroidogenic Factor 1, orphan nuclear steroid receptor
- Knockout cause lack gonad
- Mutation cause sex reversal
- Influence MIS and DAX-1 expression

**LIM1** - LIM Homeobox gene Lhx9
- Knockout cause lack gonad
- LIM knockout cause lack SF1 (? Upstream)

---

**Role of Neurotrophins in Rat Embryonic Testis Morphogenesis (Cord Formation)**

Elisa Levine, Andrea S. Cagno, and Michael K. Skinner

Center for Reproductive Biology, Department of Genetics and Cell Biology, Washington State University, Pullman, Washington 99164-4211

**ABSTRACT**

The process of seminiferous cord formation is the first morphological event that differentiates a testis from a more androblastoma-like tissue. Cord formation occurs by seminiferous epithelium being squeezed out to the periphery of the testis. To investigate the role of neurotrophins in cord formation, the expression of two neurotrophin receptors, TrkB and TrkC, were studied by immunohistochemistry during the first 2 weeks of testicular development. In rat testes, both TrkB and TrkC receptors were expressed in all stages of cord development, and in a stage-specific manner appeared around developing cords at E14 in the extraembryonic testes. In both, staining for p75NTR was localized to a single type of cell, i.e., the peritubular cells that are involved in the extension of amniotic testis cord formation.
A. The X and Y chromosomes

B. Discussing SPY

1. Shown region
2. 389 concerning region
3. Break point

C. XF analysis

1. Autosomal point
2. Fragment point

D. Promoter for sperm preservation and central nervous system

E. Promoter for germ cells

Figure 1: An evolving understanding of sex. In humans, sex is based on the presence or absence of the Y chromosome, which has a much larger partner, X. The testis-determining factor (TDF) that drives male development was known to be on the short arm of the Y, but its identity was a mystery. In 1990, Steinke et al. found that a region with only a small piece of Y, which had been broken and placed in the X. They covered the X and Y with a 50-kb base pair between the break points and the region at the tip of the Y that is shared with the X. Initially, several regions (red) that were specific to the Y. One of these regions contained the TDF gene. This discovery led to an understanding of how X and Y evolved. The gene SPY was located on a region of the chromosome that is conserved in the ancestors of mammals. A promoter sequence drives expression of SPY in sperm precursors and the central nervous system. The promoter on one copy of SPY was replaced with a sequence that drives expression in the undifferentiated germ cells (those that can develop into either an ovary or a testis). This expression pattern allowed the new gene, SPY, to direct development. Over time, genes not needed for male development were degraded on the chromosome, giving rise to the X (partly adapted from ref. 1).
**FIG. 1.** Comparison of the human and mouse SRY protein domains. Shown are the protein domains of the human and mouse SRY proteins, showing the conserved HMG domain (HMG) and the glutamine-rich domain present in mice but absent in humans.

**FIG. 2.** A model of the structure of the SRY HMG box bound to DNA. The nuclear magnetic resonance structure of the SRY HMG domain, showing the three alpha-helices and the L-shaped conformation and binding to the minor groove of DNA causing it to bend and severely unwind (35).
**Figure 1. Molecular Pathways Controlling Sex Determining in XY and XX Mammals**

Positive regulation is indicated by an arrow, whereas inhibition is represented by a blunt-ended line. The nature of the regulatory relationship between Sry and Sox9, represented by a dotted arrow, is not yet clear. Some of the unknown elements are indicated by question marks.

**XX**  
WNT4 → β-catenin → Ovary

**XY**  
SRY → β-catenin → Ovary

SRY → SOX9 → Testis
SOX4 regulates gonad morphogenesis and promotes male germ cell differentiation in mice. 
Zhao L, Arsenault M, Ng ET, Longmores E, Chou TC, Harting E, Koopman P. 

**Abstract**

The group C SOX transcription factors SOX4, -11 and -12 play important and mutually overlapping roles in development of a number of organs. Here, we examined the role of SoxC genes during gonadal development in mice. All three genes were expressed in developing gonads of both sexes, predominantly in somatic cells, with Sox4 being most strongly expressed. Sox4 deficiency resulted in elongation of both ovaries and testes, and an increased number of testis cords. While female germ cells entered meiosis normally, male germ cells showed reduced levels of differentiation markers Nanos2 and Dnmt3l and increased levels of pluripotency genes Cripto and Nanog, suggesting that SOX4 may normally act to restrict the pluripotency period of male germ cells and ensure their proper differentiation. Finally, our data reveal that SOX4 (and, to a lesser extent, SOX11 and -12) repressed transcription of the sex-determining gene Sox9 via an upstream testis-specific enhancer core (TESCO) element in fetal gonads, raising the possibility that SOXC proteins may function as transcriptional repressors in a context-dependent manner.
Sex Determination in the Mammalian Germline.
DMRT1 protects male gonadal cells from retinoid-dependent sexual transdifferentiation.


DMRT1 directly represses transcription in Sertoli cells of potential feminizing genes including Foxl2, Esr2, and the Wnt/β-catenin pathway genes Wnt4 and Rspo1 (Matson et al., 2011). This paper shows that DMRT1 thereby allows Sertoli cells to produce RA that is necessary for spermatogenesis without causing RARα to activate these feminizing genes, which also activate one another. The model also indicates that it is possible, based on data from other systems, that RARα synergizes with products of some of the feminizing genes to drive transdifferentiation. In addition to the genes shown, DMRT1 also represses Cyp19a1/aromatase, which makes estradiol that stimulates ER activity (Matson et al., 2011).
A major event in mammalian male sex determination is the induction of the testis determining factor Sry and its downstream gene Sox9. The current study provides one of the first genome wide analyses of the downstream gene binding targets for SRY and SOX9 to help elucidate the molecular control of Sertoli cell differentiation and testis development. A modified ChIP-Chip analysis using a comparative hybridization was used to identify 71 direct downstream binding targets for SRY and 109 binding targets for SOX9. Interestingly, only 5 gene targets overlapped between SRY and SOX9. In addition to the direct response element binding gene targets, a large number of atypical binding gene targets were identified for both SRY and SOX9. Bioinformatic analysis of the downstream binding targets identified gene networks and cellular pathways potentially involved in the induction of Sertoli cell differentiation and testis development. The specific DNA sequence binding site motifs for both SRY and SOX9 were identified. Observations provide insights into the molecular control of male gonadal sex determination.

The cascade of molecular events involved in mammalian sex determination has been shown to involve the SRY gene, but specific downstream events have eluded researchers for decades. The current study identifies one of the first direct downstream targets of the male sex determining factor SRY as the basic-helix-loop-helix (bHLH) transcription factor TCF21. SRY was found to bind to the Tcf21 promoter and activate gene expression. Mutagenesis of SRY/Sox9 response elements in the Tcf21 promoter eliminated the actions of SRY. SRY was found to directly associate with the Tcf21 promoter SRY/Sox9 response elements in vivo during fetal rat testis development. TCF21 was found to promote an in vitro sex reversal of embryonic ovarian cells to induce precursor Sertoli cell differentiation. TCF21 and SRY had similar effects on the in vitro sex reversal gonadal cell transcriptomes. Therefore, SRY acts directly on the Tcf21 promoter to in part initiate a cascade of events associated with Sertoli cell differentiation and embryonic testis development.
Summary of SRY downstream genes.
Proposed downstream actions of SRY on Sox9 and Tcf21 genes, along with Clbn4, Ntf3, and others yet to be identified. TCF21 induction of Sertoli cell differentiation and expression of marker genes such as Amh indicated. Combined actions of SRY and SF1 on Sox9 expression and actions on Fgf9 and Pgds expression indicated.


TCF21 binding target gene functional categories. Total numbers of target genes associated with a specific category are presented on the y-axis and gene functional categories on the x-axis.

Schematic diagram of the hypothesized cascade of bHLH transcription factors involved in Sertoli cell differentiation and gonadal sex determination.
Sex Differentiation
Role Testosterone -

1) Wolffian Duct development
2) Male Reproduction Genitalia
3) External Genitalia
Sex Determination in Other Species

Sex chromosome differentiation. A. Reconstructed evolutionary path of sex chromosome differentiation in humans. Sex chromosomes originate from autosomes that acquired a sex-determining function (the Sry gene) after their split from monotremes. Suppression of recombination between the sex chromosomes, associated with degeneration of the non-recombining region of the Y chromosome, results in the morphological and genetic differentiation of sex chromosomes. Recombination suppression occurred in multiple episodes along the human X and Y chromosome, forming so-called evolutionary strata. The oldest stratum is shared between eutherian mammals and marsupials, while the youngest stratum of humans is primate-specific. B. The degree of sex chromosome differentiation ranges widely across species, spanning the entire spectrum of homomorphic to heteromorphic sex chromosomes, from a single sex-determining locus, as seen in pufferfish, a small differentiated region (strawberry and emu), most of the sex chromosomes apart from short recombining regions (humans), to the entire sex chromosome pair, as seen in Drosophila. Note that the sex chromosomes are not drawn to scale.

Diversity of sex determination systems for representative plant and animal clades. The bubble insert graph for the plant clades represents the relative proportion of species with documented sex chromosomes within plants with separate sexes. Vertebrates: Mammalia (placental, marsupial, and monotreme mammals), Aves (birds), Reptilia (turtles, snakes, crocodiles, lizards), Amphibia (frogs, ...
Cocrystal structure of the quaternary DMRT1–DNA complex. Three α-helices intrude into the major groove, two of them (purple and green) in the same region and the third α-helix (cyan) a helical turn away. The zinc-binding modules bind the minor groove (shape readout). In addition, protein–protein contacts are formed between the three DM domains (cooperativity).

Top: temperature-dependent sex determination and corresponding embryonic developmental stages in the red-eared slider turtle (Trachemys scripta). Bipotential embryonic gonads contain primordial germ cells (PGC) and differentiate to either ovary (at 31°C, FPT) or testis (at 26°C, MPT) (From Ramsey et al., 2007). Bottom: embryonic development from stage 14 to stage 25 in the red-eared slider turtle (adopted from Greenbaum, 2002).

foxl3−/− XX gonads produce functional sperm.

(A) Fertilization rate of artificial insemination by using sperm from wild-type testes (n = 3) and foxl3−/− ovaries (n = 8). In each artificial insemination, one gonad was used. (B) Hatching rate of the fertilized eggs in (A). (C) Unfertilized eggs. (D) Eggs fertilized by sperm derived from foxl3−/− ovaries. The activated egg membrane is shown in (D'). (E) Embryos hatched from the eggs in (D). Statistics by two-tailed Student’s t test; *P < 0.05.

Table 1. Known master sex-determining genes in vertebrates and insects, and their paralogs.

<table>
<thead>
<tr>
<th>Species</th>
<th>Master sex-determining gene</th>
<th>Sex-determining mechanism</th>
<th>Gene paralog</th>
<th>Parathyroid function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>zebrafish</td>
<td>diY</td>
<td>sex-determining Y</td>
<td>diF</td>
<td>inhibitory</td>
<td>[111]</td>
</tr>
<tr>
<td>chicken (Gallus gallus)</td>
<td>direct</td>
<td>sex-determining Z</td>
<td>50</td>
<td>sex-determining transcrip</td>
<td>[112]</td>
</tr>
<tr>
<td>chicken (Gallus gallus)</td>
<td>dose-dependent Z</td>
<td>sex-determining transcrip</td>
<td>[113]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African clawed frog (Xenopus laevis)</td>
<td>direct</td>
<td>sex-determining transcrip</td>
<td>[113]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nematode (C. elegans)</td>
<td>direct</td>
<td>sex-determining transcrip</td>
<td>[114]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African clawed frog (Xenopus laevis)</td>
<td>direct</td>
<td>sex-determining transcrip</td>
<td>[114]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smooth tongue sole (Cryptobranchus amboinensis)</td>
<td>direct</td>
<td>sex-determining transcrip</td>
<td>[114]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zebrafish (Group II)</td>
<td>50</td>
<td>sex-determining transcrip</td>
<td>50</td>
<td>sex-determining transcrip</td>
<td>[114]</td>
</tr>
<tr>
<td>Human (Homo sapiens)</td>
<td>P</td>
<td>sex-determining W</td>
<td>50</td>
<td>sex-determining transcrip</td>
<td>[117]</td>
</tr>
<tr>
<td>African clawed frog (Xenopus laevis)</td>
<td>direct</td>
<td>sex-determining W</td>
<td>50</td>
<td>sex-determining transcrip</td>
<td>[117]</td>
</tr>
<tr>
<td>Chinese hamster (Cricetulus griseus)</td>
<td>direct</td>
<td>sex-determining W</td>
<td>50</td>
<td>sex-determining transcrip</td>
<td>[117]</td>
</tr>
<tr>
<td>Human (Homo sapiens)</td>
<td>P</td>
<td>sex-determining W</td>
<td>50</td>
<td>sex-determining transcrip</td>
<td>[117]</td>
</tr>
<tr>
<td>African clawed frog (Xenopus laevis)</td>
<td>direct</td>
<td>sex-determining W</td>
<td>50</td>
<td>sex-determining transcrip</td>
<td>[117]</td>
</tr>
<tr>
<td>Chinese hamster (Cricetulus griseus)</td>
<td>direct</td>
<td>sex-determining W</td>
<td>50</td>
<td>sex-determining transcrip</td>
<td>[117]</td>
</tr>
</tbody>
</table>


Overview of the evolutionary relationship of the fem gene and copies (fem1, csd, tra) in social insect species.