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Introduction: vertebrate sex determination and gonadal differentiation

G. Scherer

Institute of Human Genetics and Anthropology, University of Freiburg, Breisacherstr. 33, D-79106 Freiburg (Germany), Fax + 49 761 270 7041, e-mail: scherer@humangenetik.ukl.uni-freiburg.de

Male and female mammalian embryos are morphologically indistinguishable during early development. Then, at a specific developmental stage, the bipotential gonadal anlagen differentiate into testes when the chromosomal sex of the embryo is XY, and of the two duct systems laid down in either sex, the Wolffian (mesonephric) duct differentiates into the male internal genitalia while the Müllerian (paramesonephric) duct regresses. When the chromosomal sex of the embryo is XX, the bipotential gonadal anlagen develop into ovaries rather than testes, the female internal genitalia form from the Müllerian duct, and the Wolffian duct regresses. In his famous castration experiments on rabbit embryos in the late 1940s [1], Alfred Jost discovered that if the gonads from male embryos were removed before sexual differentiation occurred, the fetus develops into a female. These and subsequent experiments led him to the conclusion that fetal testes secrete two hormones, one designed to regulate the formation of male internal genitalia from the Wolffian duct, the other to cause the regression of the Müllerian ducts that would otherwise become female internal genitalia. It was later shown that the former process is regulated by a steroid hormone, testosterone, secreted by testicular Leydig cells, while the latter process results from the action of a peptide hormone known as AMH (anti-Müllerian hormone) or MIS (Müllerian-inhibiting substance), produced very early in testis differentiation from Sertoli cells. Jost's experiments also led to the concept that sex determination in mammals is equivalent to testis determination, and that a testis-determining factor must reside on the mammalian Y chromosome. The gene encoding this factor was finally identified in 1990 as *SRY* [2].

Like mammals, all other vertebrates go through a sex-neutral stage during early embryonic development, but there are important differences in how gonadal sex is determined and early gonadal differentiation is regulated. Birds also have a genetic sex-determining mechanism, but here it is the female that is the heterogametic sex (ZW), whereas the male is the homogametic sex (ZZ). Reptiles, amphibians and fish exhibit different mechanisms of sex determination: genotypic sex determination of both the XX/XY and ZZ/ZW type whereby the sex chromosomes are either heteromorphic, as in mammals and birds, or homomorphic, identifiable only by genetic means; and environmental (phenotypic) sex determination such as temperature-dependent sex determination, most intensely studied in oviparous reptiles. Another significant difference between mammals and the other vertebrate classes is that in the latter, gonadal development is under the influence of sex steroid hormones, and steroid-induced sex reversal is extensively studied in nonmammalian vertebrates.

Half a century after the seminal discoveries of Alfred Jost, and almost a decade after the isolation of the Y-chromosomal testis-determining gene *SRY*, it now seems a particularly appropriate time point to summarize our present knowledge and concepts in the field of sex determination and early gonadal differentiation. This is what this collection of reviews tries to provide, not only restricted to sex determination in mammals, but covering this topic in the entire phylum of vertebrates.

Susumu Ohno has been an influential figure in many areas of biology, not least in the field of vertebrate sex determination. His monograph on sex chromosomes and sex-linked genes [3] is a classic in the field. In his opening review, he considers sex-determining genes in a broader context, drawing from information emerging from the ongoing genome projects in human and other species. These projects corroborate his long-held view that the genome of gnathostomic vertebrates (vertebrates with jaws) underwent two rounds of te-

traploidization and thus contains four times as many genes as the invertebrate genome. Applying this 'one-to-four rule', as he calls it, to genes pertinent to vertebrate gonadal development and differentiation leads him to a number of stimulating insights and to paralogous genes that may be worth studying in the future. Ohno also surprises us with his unorthodox view that *SRY* may have an ancient origin, having become extinct in early vertebrates and resurrected in the mammalian ancestor.

The three reviews that follow deal in detail with *SRY* and four other genes—*WT1*, *SF-1*, *SOX9* and *DAX-1*—that have been identified over the last years as essential factors for the development of the gonadal anlagen and their differentiation into gonads. They all encode transcription factors. Keith Parker and his coauthors review the studies that led to the isolation of *WT1* (Wilms tumour suppressor 1), a tumour suppressor gene implicated in the formation of embryonic kidney tumours, and of *SF-1* (steroidogenic factor 1), a key regulator of steroidogenic tissues. They also review evidence from human XY sex-reversal syndromes for a function of *WT1* in early testis development. They finally discuss various models of how WT1 and SF-1 might functionally interact in the regulation of the *MIS* gene, and how DAX-1 might interfere in this regulation.

DAX-1 itself is reviewed by Peter Goodfellow and Giovanna Camerino. Camerino's group has isolated the *DAX-1* gene from a region of the short arm of the human X chromosome that, if duplicated, causes male-to-female sex reversal, overriding the testis-inducing action of *SRY*. This effect has been replicated by *Dax-1* overexpression in transgenic mice, implicating *DAX-1* in sex determination as an 'anti-testis gene'. DAX-1 is also involved in adrenal function, as indicated by patients with DAX-1 deficiency who suffer from adrenal hypoplasia. The DAX-1 protein, like SF-1, is an orphan nuclear homone receptor that seems to act as a transcriptional repressor. DAX-1 is present in many tissues together with SF-1, with which it directly interacts, as reviewed here.

Since 1994, *SRY* has to share its role as a mammalian testis-determing gene with *SOX9*, an autosomal gene. SOX9 is a distant evolutionary relative of SRY by way of a common DNA binding motif known as an HMG domain. Other than *SRY*, the only known function of which is to act as a 'dominant' (correctly, epistatic) testis inducer, *SOX9* has two functions, as a regulator of both testicular and chondrogenic development. Peter Koopman brings us up to date on what has been learned about these two genes. This is deceptively little as regards *SRY*. While its testis-inducing function is indisputable since the now classic transgenesis experiments in the mouse, this function is restricted to eutherian mammals and, possibly, to marsupials. No clear *SRY* orthologue has been identified in other vertebrates, and the true target gene for *SRY* is still elusive. In contrast, for *SOX9*, besides the *COL2A1* collagen gene, there is recent evidence for *MIS* as a possible target gene. While *SRY* is a rapidly evolving gene, *SOX9* is highly conserved and appears to function as a testis-determination or -differentiation factor also in nonmammalian vertebrates. Peter Koopman sketches out a scheme that incorporates *SRY*, *SOX9* and the other known factors in sex determination in a combinatorial network.

Andrew Pask and Jenny Marshall Graves look at sex determination from the Australian perspective, focussing on the marsupials and monotremes. Like the placental mammals, these two other groups of the class Mammalia also have an XX/XY genetic sex-determining mechanism. Fittingly, an *SRY* homologue has been identified on the marsupial Y chromosome. However, definitive proof for *SRY* as a testis-inducing factor in marsupials is still lacking. Also, no evidence for a Y-chromosomal *SRY* homologue in monotremes exists. These authors thus argue that *SRY* may have evolved its testis-determining function for the placental mammals only, possibly a mere 80 million years ago, having been derived from the X-chromosomal *SOX3* gene.

Two models exist for the genetic mechanism by which sex is determined in birds. One argues for the existence of an ovary-inducing gene on the female-specific W chromosome. The other assumes a dosage-dependent mechanism, where an autosomal factor interacts with a single dose (ZW) or a double dose (ZZ) of a Z-linked gene product. As Michael Clinton and L. Haines point out, the issue remains unresolved, as no reliable reports on birds with informative genotypes such as ZZW or Z0 exist. With the exception of *SRY*, and to date *DAX-1*, chick homologues to the other mammalian genes mentioned above have been identified and are under intensive study, revealing a high degree of conservation of the molecular mechanisms involved in gonadal development. From sex steroid-induced sex reversal and other evidence in the chicken, the involvement of steroid hormones in gonadal development in birds has been known for some time. Recent evidence supporting this view is discussed in this review.

Temperature-dependent sex determination (TSD) is a widespread phenomenon in reptiles. In TSD reptiles, masculinizing temperatures yield 100% or a majority of males, whereas feminizing temperatures yield 100% or a majority of females, whereas at intermediate temperatures, males and females are produced. While there is no solid evidence yet for the true nature of the temperature target, as Claude Pieau, one of the pioneers in the field, and his colleagues outline in their review, it is now well established that temperature, directly or indi-

rectly, influences the synthesis of aromatase. This enzyme complex that converts androgens to estrogens is a keyplayer in ovary differentiation during the thermosensitive period (TSP). Pieau and colleagues illustrate the involvement of estrogens in ovary differentiation by their own experimental series in the European freshwater turtle *Emys orbicularis*, where treatment with estrogens before or during the TSP induced ovary differentiation at male-producing temperatures, whereas treatment with antioestrogens or aromatase inhibitors resulted in testis differentiation at female-producing temperatures. They conclude their review by summarizing recent molecular studies dealing with the cloning and analysis of reptilian homologues for mammalian *AMH*, *DAX-1*, *SF-1*, *WT1* and *SOX9*, and present a model for the possible interactions of these genes with the aromatase gene in TSD reptiles.

Amphibians have a genetic mechanism of sex determination, of both the XX/XY and ZZ/ZW type. Although the W chromosome is known to carry an epistatic feminizing factor in some species, as shown by triploid ZZW females, the molecular nature of this factor and how it operates are obscure. Because this genetic mechanism of sex determination can easily be overridden by environmental (temperature) and hormonal (sex steroid) influences, and even occurs spontaneously in nature, experimental sex reversal has been extensively studied in amphibians and thus is the major focus of the review by H. Wallace and colleagues. They illustrate such experimental sex reversal by their own unpublished studies on the crested newt *Triturus cristatus* with an XX/XY system, where application of high temperature resulted in sex-reversed XX males, whereas application of low temperatures produced sex-reversed XY females, which could also be produced by application of estradiol.

The final review by Jean-François Baroiller and his colleagues covers gonadal sex differentiation in fish, which again display male (XY) and female (ZW) heterogamety, sometimes even in the same species. Because nothing is yet known on what the genes are that are primarily involved in sex determination, with molecular studies only beginning, their review has its focus on their field of expertise, the endocrine and environmental control of gonadal sex differentiation. As they discuss, there is enormous plasticity in gonadal development in fish, where gonadotropins, sex steroids, temperature and even social factors, a phenomenon in vertebrate sex determination unique to fish, can all influence gonadal differentiation and may even lead to complete sex reversal.

Together, these reviews document an impressive body of knowledge on vertebrate sex-determining mechanisms accumulated since the pioneering work of Alfred Jost in mammals. At the same time, these reviews also show how incomplete the picture still is, and that our ignorance in many areas is after all rather deep. The handful of genes known to be involved in gonadal developmental, first identified in mammals and now shown, except for *SRY*, to fulfill similar roles in non-mammalian vertebrates, are of course not the entire story. The human geneticist is constantly reminded of the existence of additional, still-to-be-discovered genes that are at fault in XY sex-reversal cases where mutations in *SRY* and other known genes have been excluded [4]. A few interesting new candidates are already known and deserve further study [5–7]. Others can be expected to come to light by their interaction with already known components, by positional cloning approaches or by harvesting genome information by way of paralogy, as outlined by Ohno in his review.

In addition to finding the missing pieces in the puzzle, future work needs to address other unanswered issues, such as the factor(s) that regulate gonadal *SRY* expression in mammals and the target of SRY action; the nature of ovary-determining genes in mammals—as yet an entirely virgin field; the issue whether sex in birds is determined by Z chromosome dosage or by an ovary-determining factor on the W chromosome, and the cloning of the responsible gene(s), also in other vertebrates with a ZZ/ZW system; the precise molecular mechanism of the temperature switch in TSD vertebrates; and the effector genes and proteins involved in steroid-regulated gonadal development. Research to clarify these and other open issues in vertebrate sex determination and gonadal differentiation, a developmental process of fundamental importance, is guaranteed to keep many of us busy and fascinated for a long time to come. If the articles collected in this multi-author review can stimulate this quest, so much the better.

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