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DMRT1/Dmrt1, the Sex Determining or Sex Differentiating Gene in Vertebrata

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Although the phenomenon of sexual dimorphism is widespread in vertebrates, the molecular mechanism of sex-determination is not the same across animal phyla, in contrast to other areas of developmental biology. Recent extensive studies, however, have given proof of evolutionarily conserved function in genes which share a novel DNA binding DM domain, primarily identified in two invertebrate sex regulatory genes: *doublesex* of *Drosophila melanogaster* and *mab-3* of *Caenorhabditis elegans.* Their mammalian autosomal homologue, *DMRT1*, first isolated in humans, was further discovered in genomes of various vertebrate species and appears to be involved in similar aspects of sexual development. Its precise role is still speculated, thus identification of sex reversal mutations, functional studies as well as determination of the sex-specific expression profile during embryogenesis are still being undertaken. Is this a sex determining rather than a sex differentiating gene? Is it involved in a dosage-sensitive mechanism? On what level does it function in the hierarchy of the sexual regulatory gene cascade? Recent results are discussed in this paper.

Key words: *DMRT1*, *Dmrt1*, sex determination, sex differentiation, vertebrate sexual development.

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The mechanisms leading to the development of sexual dimorphism vary remarkably between animal species, not only between phyla, but even in a single phylum. Vertebrates reflect these differences on the level of both switch mechanisms used in sex determination and the genes responding to these switch signals and involved in molecular sex regulatory pathways. Primary sex-determining signals can be either genetic or environmental. The known genetic signal is the presence of the Y chromosome in the heterogametic XY male sex in mammals and some fish or the as yet unknown genetic factor in the heterogametic ZW female sex in birds and some reptiles. The initial environmental cues include temperature (turtles and alligators), social factors (some fish) and population density (ZARKOWER 2001). Despite this diversity, all these sex-determining systems control the fate of bipotential embryonic gonads, i.e. they determine whether the gonads undergo testis or ovary development. Gonads do not only contain germ cells but also secrete hormones that conscript the body into sexual differentiation. Several genes are required for gonadal sexual development, the process of which might be divided into three steps: initial formation of the sexually indifferent gonads, the critical sex-determining period and gonad differentiation after sex determination.

The cascade of sex regulatory genes that act during gonadogenesis is best established in mammals (Fig. 1). The crucial gene for primary sex determination in most eutherian mammals is *SRY/Sry* (the sex-determining region on the Y chromosome), the heterosomal dominant male-determining locus which is responsible for testis formation from ambiguous XY gonads (SINCLAIR *et al*. 1990; KOOPMAN *et al.* 1991). This sufficient testis-determining factor seems not to be highly conserved among vertebrates due to its absence in non-mammalian animals. In birds, where sex is genetically determined (ZZ/ZW system), it still remains unknown whether the primary switch gene is a W-linked female dominant factor or a dosage-sensitive gene residing on the Z chromosome and triggering testis differentiation.

The autosomal gene *SOX9/Sox9* (*Sry*-box containing gene 9), related to *SRY/Sry*, is postulated to be the immediate downstream target of *SRY/Sry* in

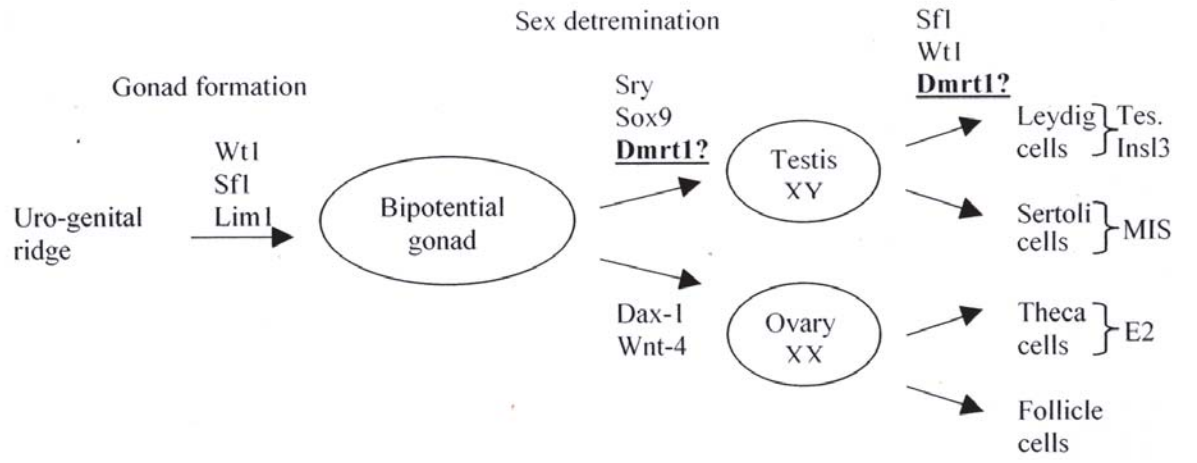

Fig. 1. Sex determination in mammals. Mammalian mechanism of sexual development is initiated during embryogenesis and is associated with processes of gonad formation, their sex determination and their further differentiation into testis in male XY embryo and ovary in female XX embryo. Each of these processes requires activity of several genes. *Wt1* (Wilms tumour 1), *Sf1* (steroidogenic factor 1), *Lim1* (LIM homeobox protein 1) act during gonad formation from mesoderm of the uro-genital ridge. *Sry* (sex-determining region on the Y chromosome) and *Sox9* (*SRY*-box containing gene 9) are expressed in the critical period of sex determination after which testis differentiation occur. No equivalent of the *Sry* gene was found in the XX embryo, but *Dax-1* (nuclear receptor subfamily 0, group B, member 1) seems to antagonize male gonad development and acts with another gene, *Wnt-4* (wingless-related MMTV integration site 4). *Sf1* and *Wt1* are also required for differentiation of somatic testis cells into Leydig cells that secrete testosterone and Insl3 (insulin-like 3) and into Sertoli cells producing MIS (Müllerian inhibiting substance), that suppresses ovary differentiation. The ovarian theca cells secrete oestrogen (E2) directing the differentiation of the female embryo. No clear evidence univocally supports the role of *DMRT1* (*dsx* and *mab-3*-related transcription factor 1) in male sex development in mammals (it may be activated before the onset of testis differentiation or after this event).

mammals and both *SRY/Sry* and *SOX9/Sox9* are necessary for male sex determination in this animal taxon (KOOPMAN 1999). However, unlike *SRY/Sry*, *SOX9/Sox9* plays an important role in male sexual development not only in mammals but also in birds (KENT *et al*. 1996) and reptiles (SPOTILA *et al*. 1998). Although it is upregulated in these vertebrate species, the expression of avian and reptilian *Sox9* occurs after the onset of sexual differentiation, thus these homo- logues cannot be considered as primary sex-determining genes.

Do the vertebrates that lack *Sry* contain other switch gene(s) necessary for testis development during the critical sex determining period? Do mammals require other gene(s) for the unaffected developmental mechanism determining sexual dimorphism? This review indicates a new candidate gene that partly fills the gap in some vertebrate molecular sex-determining pathways.

DMRT1, a highly conserved gene in Vertebrata

Among genes involved in sexual development, *DMRT1* (*doublesex* and *mab-3* related transcription factor 1) is the only one found to date to be so far conserved between phyla. This evolutionary conservation refers not only to its nucleotide and protein sequence similarity, but also to its function. *DMRT1* contains the strongly conserved DM domain, a new zinc finger-like DNA-binding motif, first identified in the *doublesex* gene in *Drosophila melanogaster* (ERDMAN & BURTIS 1993) and further in the *mab-3* gene in *Caenorhabditis elegans* (RAYMOND *et al.* 1998). The role of these two invertebrate downstream sex regulatory genes in somatic sex determination and differentiation is well characterized (BURTIS & BAKER 1989; SHEN & HODGKIN 1988). Both genes control analogous aspects of sexual development: regulation of yolk protein gene transcription (YI & ZARKOWER 1999), differentiation of male-specific sense organs (SHEN & HODGKIN 1988, Yi *et al.* 2000) and mediation of male mating behaviour (YI *et al.* 2000). Furthermore, they can be functionally interchangeable *in vivo* (RAYMOND *et al.* 1998). *DMRT1*, a human male regulatory gene related to invertebrate *dsx* and *mab-3*, is the first DM domain putative transcription factor discovered in a vertebrate genome (RAYMOND *et al.* 1998). The genomic organization of the gene (RAYMOND *et al.* 1999a), chromosomal mapping studies (RAYMOND *et al.* 1998), cytogenetic diagnosis and mutational screening of patients with sexual abnormalities (RAYMOND *et al.* 1999a; MUROYA *et al.* 2000; ÕUNAP *et al*. 2004) and analysis of expression profiles of the gene in both adult tissues (RAYMOND *et al.* 1998), and embryo sections (MONIOT *et al.* 2000), have provided significant data on the role of human *DMRT1* in male sexual development and

its homology to sex regulatory genes in Nematoda and Antropoda. To better understanding the function of *DMRT1* in the process of testicular differentiation in humans and to support its evolutionarily conserved status in the animal kingdom, these studies have been extended to other vertebrate species. Until now *Dmrt1* has been identified from mammals (mouse, rat), birds (chicken), reptiles (alligator, turtle, lizard), amphibians (frog) and fish (RAYMOND *et al.* 1999b; CHEN & HECKERT 2001; SHAN *et al.* 2000; SMITH *et al.* 1999; KETTLEWELL *et al.* 2000; TORRES-MALDONADO *et al.* 2002; MURDOCK & WIBBELS 2003; SREENIVASULU *et al.* 2002; SHIBATA *et al.* 2002; BRUNNER *et al.* 2001; MATSUDA *et al.* 2002; MARCHAND *et al.* 2000; GUAN *et al.* 2000; GUO *et al.* 2005). Sequence comparisons of various vertebrate *Dmrt1* genes with human and invertebrate homologues and their chromosomal locations and embryonic expression profiles may indicate an implication in the first universal mechanism of sex determination across animal phyla.

All of these orthologues exhibit a high level of sequence identity with human *DMRT1* (from 98% in mice to about 87% in fish at the amino acid level), but only inside a single DM domain that is present near the N-terminus. Outside the DM domain there is also another conserved proline/serine rich region at the C-terminus in both the majority of vertebrate *Dmrt1* genes and the invertebrate *mab-3* gene.

Human *DMRT1* maps to autosomal locus 9p24.3, the critical deleted region, that, when in monosomy, manifests gonadal dysgenesis and male-to-female sex reversal in XY individuals. Murine *Dmrt1* is localized on the central region of chromosome 19 which displays conserved synteny with human distal chromosome 9p. Furthermore, the Z-linked chicken *Dmrt1* and human *DMRT1* are also at comparable chromosomal locations because of the extensive conserved synteny between chicken Z sex chromosome and human chromosome 9 including 9p24.3 (NANDA *et al.* 1999).

However, the strongest evidence for conserved *DMRT1*/*Dmrt1* function in testicular differentiation comes from expression studies. In all of the examined vertebrates, *DMRT1*/*Dmrt1* appears to have a gonad-specific and sexually dimorphic expression profile during embryogenesis. However, the same studies have also revealed some differences in the spatial and temporal expression of *DMRT1*/*Dmrt1* gene derived from various vertebrate species. In most of the cases it is up-regulated either late during sex-determination or during the early testis-differentiation period. Thus there is little doubt that *DMRT1*/*Dmrt1* is a versatile vertebrate locus involved exclusively in early male gonad formation.

Dmrt1 as the sex-determining gene

The discovery of the conserved role of *DMRT1*/*Dmrt1* in vertebrate testis development allows for the consideration of *DMRT1*/*Dmrt1* as a switch sex-determining gene, especially in animals not containing *Sry* gene. This presumption seems to be true in reptiles employing a temperature-dependent sex determining strategy and to chicks with a genetic ZZ/ZW sex-determining mechanism. *Dmrt1* is the earliest known gene whose expression is considerably higher in the genital ridge of developing male gonads than in the undifferentiated ovary in these vertebrates. In three of the examined reptilian species with temperature-depending sex determination, *Alligator mississippiensis*, *Trachemys scripta, Lepidochelys olivacea*, the mRNA level of *Dmrt1* was higher in embryos incubated in a male-producing temperature than in embroys incubated in a female-producing temperature (SMITH *et al.* 1999, KETTLEWELL *et al.* 2000, TORRES-MALDO- NADO *et al.* 2002). ZARKOWER suggests that temperature-sensitive expression of *Dmrt1* in reptiles may be regulated either by an unknown temperature-depending genetic factor or by itself in the process of autoregulation (ZARKOWER 2001). The second postulate is more consistent with the primary sex determining function of *Dmrt1* in these vertebrate species. In addition, studies in chicken embryos indicate this role for *Dmrt1* in male development (RAYMOND *et al.* 1999b, SMITH *et al.* 1999, SHAN *et al.* 2000). Expression of avian orthologues becomes sexually dimorphic before the onset of sex differentiation, stronger in developing male than in female gonads. What is more, in the later stages of embryonic development, ovarian *Dmrt1* expression becomes progressively weaker and in consequence declines, in contrast to the successive and exclusively higher testicular *Dmrt1* expression continued in adult ZZ gonads. These results can be explained by the facts that chicken *Dmrt1* maps to the Z sex chromosome and, unlike the mammalian X chromosome in females, there is an absence of dosage compensation in birds. Thus, this avian putative upstream regulatory sex-determining factor seems to be involved in a dosage-sensitive mechanism. The overexpression of *Dmrt1* from two Z chromosomes in the genital ridge at the time of sex determination may be required to initiate testis differentiation, whereas one gene dosage in the bipotential ZW gonads is likely insufficient for testis formation and leads to female development. However, recent studies shed doubt on dosage dependence for *Dmrt1* in chicken because of the increased level of *Dmrt1* expression during experimentally induced female-to-male sex reversal, despite the presence of only one Z chromosome in genetically female gonads (SMITH *et al.* 2003). Nevertheless,

the involvement of a *DMRT1* dosage-sensitive mechanism in testis differentiation has also been proposed in humans with defective testis development and sex reversal in XY individuals with monosomic deletion of 9p, which may be due to haploinsufficiency for expression of this male regulatory factor (either by itself or with nearby genes) (RAYMOND *et al.* 1999a). Furthermore, the expression profile of *DMRT1* in developing human embryos is similar to the role of *Dmrt1* genes in human and avian sex development. *DMRT1* expression, together with *SRY*, was detected in the genital ridge of 6-week old XY human embryos with morphologically undifferentiated gonads and later in newly differentiating Sertoli cells of 7-week old male embryos (MONIOT *et al.* 2000). This result suggests a role for *DMRT1* in human sex determination, similar to the function of the chicken homologue as a primary sex-determining gene acting in early gonadogenesis prior to sex differentiation. Although in chicken both developing male and female gonads express *Dmrt1* at a higher level in ZZ embryos, expression of *DMRT1* appears not to be detectable in undifferentiated human female embryos at all. Some questions have to appear in the light of these results: does this male-specific gonadal expression pattern of human *DMRT1* occur in other mammalian species which either contain or lack *SRY*? Does chicken *Dmrt1* play this same active role in all birds and reptiles with a ZZ/ZW sex-determining mechanism? What is the function of *Dmrt1* homologues in lower vertebrates, do they express *Dmrt1* in a male-specific manner? Only some of these questions have been resolved so far.

Dmrt1 as a sex-differentiating gene

Although the conserved function of *DMRT1*/*Dmrt1* during mammalian sex development was demonstrated in functional studies generating transgenic mice with positive expression being under the control of porcine *DMRT1* promoter sequences (BOYER *et al.* 2002), other research has indicated some divergence. Unlike the gonadal expression profile of human *DMRT1*, the murine homologue is expressed in the genital ridge of both sexes at a similar level just before gonadal differentiation and becomes sexually dimorphic (upregulated in testis) after the activation of the *Sry* gene (RAYMOND *et al.* 1999b; SMITH *et al.* 1999; DE GRANDI *et al.* 2000). This is consistent with the role of *Dmrt1* in male gonad differentiation rather than sex determination. Furthermore, male *Dmrt1* knockout mice were found to have postnatal affecting Sertoli and germ cells (in some cases similar to the phenotype of 9p deletion patients), but not sex reversal (RAYMOND *et al.* 2000). This single reported functional data also suggests the redundant function of *Dmrt1* in ovary development due to fully fertile *Dmrt1*$^{-/-}$ female murine mutants. This well-evidenced role of *Dmrt1* as a male-promoting gene was also shown in lower vertebrates. Investigation of expression profiles on both mRNA and protein levels in the frog *Rana rugosa* showed the implication of amphibian homologue in testicular, but not ovarian differentiation (SHIBATA *et al.* 2002; AOYAMA *et al.* 2003). Expression was restricted only to the developing and adult testis and to gonads in sex-reversed animals obtained by testosterone injection into female tadpoles. Expression of vertebrate *Dmrt1* genes in a male-specific manner was also documented in teleost fish, the tilapia *Oreochromis niloticus* (GUAN *et al.* 2000), rainbow trout *Oncorhynchus mykiss* (MARCHAND *et al.* 2000) and zebrafish *Danio rerio* (GUO *et al.* 2005). Although the expression of the *rtDmrt1* gene (rainbow trout *Dmrt1*) was higher in the differentiating testis when compared to the differentiating ovary and occurs in the early stages of spermatogenesis in adult testis, similar to the testis-specific expression of *tDmrt1* (tilapia *Dmrt1*), in both of these fish species there is some association of DM domain genes with ovary development. Unlike all other examined vertebrate *DMRT1*/*Dmrt1* genes, expression of *rtDmrt1* was detected in adult female gonads. What is more, the isolation of an additional DM domain gene from tilapia, *tDMO* (tilapia DM-domain gene in Ovary), expression of which is limited to the ovary, is the first report of a female specific DM domain gene in vertebrates. Additionally, the *Dmrt1* orthologue isolated from the lizard *Calotes versicolor* (a reptile with non temperature-dependent sex determination) appears to play a double role during embryo development: first in testis differentiation by promoting proliferation and differentiation of presertoli cells, later in gametogenesis in both testis and ovary by promoting proliferation of germ cells (SREENIVASULU *et al.* 2002). In contrast to these results, two other simultaneous studies have disclosed the presence of a DM domain gene in the sex-determining region on the Y chromosome (*DMY*/*Dmrt1bY*) in the medaka fish karyotype (MATSUDA *et al.* 2002; NANDA *et al.* 2002), despite the earlier identified autosomal *Dmrt1* locus in this fish species (BRUNNER *et al.* 2001). The duplicated copy of *Dmrt1* found on the Y chromosome in its specific region (*Dmrt1bY*) in fish exhibiting a genetic sex determining mechanism (XX females, XY males), is a sex-determining gene and represents the non-mammalian vertebrate equivalent of *Sry*. This probably explains the specific expression pattern of both *Dmrt1* and *DMY* during embryogenesis in medaka fish. Unlike other vertebrate homologues, *Dmrt1* in *O. latipes* is expressed after testicular

differentiation and seems to be essential for regulation of spermatogenesis, whereas *DMY* expression occurs during male gonad differentiation and in an as yet unknown manner acquires the function of a sex-determining gene (KOBAYASHI *et al.* 2004).

In conclusion, the presented data reveal the functional diversity of *DMRT1*/*Dmrt1* in the large group of vertebrate animals, especially in the extensively examined fish. However, its role seems to be limited to the upstream or downstream testis regulators acting during animal embryogenesis. In the field of sexual development, *DMRT1*/*Dmrt1* still remains the most conserved throughout vertebrates, both in its structure and function.

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