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DOI: https://doi.org/10.5772/36274

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DM Domain Genes: Sexual and Somatic Development During Vertebrate Embryogenesis

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1. Introduction

Sex determination occurs during embryo development in Metazoans that appear as two morphologically distinct sexes. This means that there is a precise time point during embryogenesis when the initial signal starts to act and directs the development of the ambiguous embryo into male or female. What are these primary sex-determination signals? They are different in various vertebrates and can be either genetically or environmentally controlled. Once they appear, they activate the cascade of different genes that respond to these signals and regulate downstream sex-developmental events. Besides the existence of two sexes, which is virtually universal in the animal kingdom, sex-developmental strategies (both the initial signals and the cascade of regulatory genes) vary between phyla and are opposite to somatic-development strategies, which have been found to be more conservative.

Vertebrate sex determination occurs in the gonadal primordium (the genital ridge), and once it takes place, the gonads are differentiated into specific male (testes) and female (ovaries) structures that, mostly because of their hormone-secretion activity, conscript the body into further sexual differentiation (somatic sexual dimorphism).

The revolution in molecular biology technology that started over 50 years ago and continues today has allowed scientists to discover the molecular background of embryogenesis starting from the identification of single genes to the prediction of entire genomic and proteomic regulatory pathways involved in embryo development.

The group of genes that has been found as very important embryogenesis regulators encodes transcription factors, proteins that interact with DNA and regulate the expression of other genes below them in the regulatory hierarchy.

This chapter is dedicated to the fascinating story of one transcription factor family, the family of DM domain genes, which has been discovered in both vertebrate and invertebrate

genomes. They all encode the DM (*doublesex* and *mab-3*) domain, possess the highly conservative zing-finger DNA-binding motif and regulate not only sexual, but also somatic developmental pathways in animals. Here, the extensive knowledge of the biology of DM domain genes in vertebrates (from the history of their discovery in different animal genomes to their function in embryo development) is presented. Moreover, the very interesting and slightly contradictory evolutional aspect of DM domain genes is emphasised. So far, they represent the only exception during vertebrate sexual development due to their structural and functional conservation between phyla. On the other hand, the successive discovery of additional vertebrate genes with the DM domain (with their variations in number and function between species) shows how rapidly their evolution took place.

2. The discovery of DM domain genes: The chronological point of view

During the last 13 years, numerous studies of vertebrate DM domain genes have been extensively carried out. Structural analyses of these genes (their genomic organisation, sequence comparisons between species, chromosomal locations, mutational screenings of individuals with developmental abnormalities) as well as their expression profiles in both adult tissues and embryo sections together with functional studies in model organisms have been performed by different research groups all over the world. Here, I present the data that displays how our knowledge of this gene family has been increased over the past decade.

2.1 The DM domain, a link between invertebrates and vertebrates

The first report about the DM domain sequence in the vertebrate genome comes from the studies of Raymond and collaborators (Raymond et al., 1998), who have identified the human locus encoding a DM domain protein. Although the authors primarily named it *DMT1* (for the first DM domain gene expressed in testis), it is now known as *DMRT1* (*doublesex* and *mab-3* related transcription factor 1). The name of the gene reveals its structural homology to sexual regulators: *dsx* (*doublesex*) in *Drosophila melanogaster* and *mab-3* (*male abnormal 3*) in *Caenorhabditis elegans*. These two invertebrate homologs encode the conserved motif similar to the zing–finger DNA-binding domain, first described in both male DSX^M and female DSX^F isoforms of *D. melanogaster* (Erdman & Burtis, 1993) and later, simultaneously with its human homolog, in MAB-3 of *C. elegans* (Raymond et al., 1998). Raymond named this motif DM domain based on its occurrence in fly DSX and worm MAB-3 proteins.

The function of two invertebrate downstream sex regulators, *dsx* and *mab-3*, in somatic sex determination and differentiation was previously well characterised (Burtis &Baker, 1989; Shen & Hodgkin, 1988), and it was found that they are evolutionarily conserved. Both genes control analogous aspects of sexual development: direct regulation of yolk protein gene transcription (Yi & Zarkower, 1999), differentiation of male-specific sense organs (Baker & Ridge, 1980; Shen & Hodgkin, 1988; Yi et al., 2000) and mediation of male mating behaviour (Yi et al., 2000). The studies of Raymond (1998) have additionally emphasised the functional relation between these two evolutionally distinct proteins, showing that they can be functionally interchangeable *in vivo*: The fly dsx^M but not dsx^F could replace *mab-3* during the development of a transgenic *mab-3* mutant *C. elegans* male.

The report of Raymond and co-authors (1998) proved importance in the research field of animal sexual development by giving the first evidence of molecular evolutionary conservation within invertebrates as well as between invertebrate and vertebrate sexual-regulatory mechanisms.

2.2 *DMRT* – Vertebrate DM domain gene family

Although the function of the invertebrate DM domain genes *dsx* and *mab-3* in somatic sexual development was described quite broadly, only little was known about the first vertebrate homolog, *DMRT1*, at the time when Raymond's paper was published (Raymond et al., 1998). His group, however, has provided very convincing data about *DMRT1* as a good candidate gene required in humans for male development. First, it was mapped to the autosomal locus (distal short arm of chromosome 9, band 9p24.3), which has been implicated in human XY sex reversal in numerous previously published reports (Crocker et al., 1988; Bennett et al., 1993; McDonald et al., 1997; Veitia et al., 1997; Veitia et al., 1998; Flejter et al., 1998). Second, *DMRT1* was expressed exclusively in testes among 50 investigated human tissues. Further evidence for *DMRT1* as a male sexual regulator came either from the later studies of its expression in human embryos (Moniot et al., 2000) or from additional reports describing sex-reversed patients with the monosomy of 9p (Raymond et al., 1999a; Calvari at al., 2000; Muroya et al., 2000; Õunap et al., 2004; Privitera et al., 2005; Vinci et al., 2007). In the meantime, the group of Zarkower from the University of Minnesota (Raymond et al., 1999b) and the group of Sinclair from the University of Melbourne (Smith et al., 1999a) published very important data about *DMRT1* expression during mouse, chicken and alligator embryogenesis. They consistently showed that *DMRT1* is unique, in that it is expressed very early and sex specifically in the gonads of three investigated species, regardless of the sex-determining mechanism used (i.e., whether chromosomal (mouse, chicken) or environmental (alligator)). These findings suggested that DM domain genes may play a role in sexual development in a wide range of vertebrate phyla. Indeed, further studies extensively carried out in all vertebrate phyla (from mammals to fish) (Table 1) have supported this hypothesis. Moreover, they have shown the high structural similarity of *DMRT1* across species (protein sequence identity within the DM domain with human DMRT1 ranges from 98% in mice to 87% in fish) as well as the conserved sexually dimorphic pattern of its expression both during early gonadogenesis and in adult tissues (Table 2). These studies, however, needed further confirmation through, for example, functional analyses of the gene (its artificial manipulation in a model organism). For the first time, functional studies were performed in 2000 by Zarkower's group (Raymond et al., 2000), who showed that homozygous *Dmrt1*$^{-/-}$ mutant male mice fail to undergo normal postnatal testis differentiation. From this data, it was clear that *Dmrt1* is a critical regulator of testis development in the mouse.

While Zarkower's group was later mostly concentrated on mouse functional studies providing more and more interesting data about the role of DMRT1 in mammalian sex-developmental pathways (Fahrioglu et al., 2007; Kim et al., 2007a; Krentz et al., 2009; Matson et al., 2010; Murphy et al., 2010; Krentz et al., 2011; Matson et al., 2011), Sinclair and his co-workers were focused on studies in the chicken (Smith et al., 1999b; Smith at al., 2003). They were constantly looking for strong evidence for *Dmrt1* as a male dosage-sensitive sex-determination locus, previously shown to be linked to the Z chromosome (avian males are

homogametic ZZ) in the region highly homologous to human 9 chromosome bearing the *DMRT1* locus (Nanda et al., 1999; Nanda et al., 2000). Their long-term studies were finally published in 2009, providing the convincing results that *Dmrt1* is indeed required for testis determination in the chicken and supporting the Z dosage hypothesis for avian sex determination (Smith et al., 2009).

Although *DMRT1* has been studied very intensively during the last decade and its function as the sex-determination/sex-differentiation locus in a wide range of vertebrate species has been very well documented in structural, expression and functional analyses, it has always been known that *DMRT1* is not the only gene with the DM domain in the vertebrate genome. Thus, there was a strong need for further investigations.

Gene Symbol	**NCBI Reference mRNA Sequence**	**Chromosome Localisation**	**Organism**	**References**
DMRT1/DMT1	AF130728	HSA 9p24.3	*Homo sapiens*	Raymond et al., 1998 Raymond et al., 1999a
Dmrt1	NM_015826.5 AL133300	MMU 19C2-C3	*Mus musculus*	Raymond et al., 1999b De Grandi et al., 2000
Dmrt1	AF379608	RNO 1q51	*Rattus norvegicus*	Chen & Heckert, 2001
Dmrt1	NM_001078060.1	BSA 8q17	*Bos taurus*	Bratuś et al., 2009 Bratuś & Słota; 2009
Dmrt1	AF216651	SSC 1q21	*Sus scrofa domestica*	Bratuś & Słota, 2009
Dmrt1	ENSMEUT00000011422*	MEU 3p	*Macropus eugenii*	Pask et al., 2003 El-Mogharbel et al., 2005
Dmrt1	AJ744848 (exon 1) AJ744847 (exon 3)	OAN X5q	*Ornithorhynchus anatinus*	El-Mogharbel et al., 2007
Dmrt1	NM_001101831.1	GGA Zp21	*Gallus gallus*	Nanda et al., 1999
Dmrt1	-	DNO Zp	*Dromaius novaehollandeae*	Shetty et al., 2002
Dmrt1	AB272609	autosom	*Rana rugosa*	Shibata et al., 2002 Aoyama et al., 2003
Dmrt1	AB201112	autosom	*Xenopus leavis*	Osawa et al., 2005 Yoshimoto et al., 2006
DM-W	AB259777	XLE W	*Xenopus leavis*	Yoshimoto et al., 2008
Dmrt1	AY316537	-	*Trachemys scripta*	Murdock & Wibbels, 2003
Dmrt1	AF335421	-	*Lepidochelys olivacea*	Torres-Maldonado et al., 2002
Dmrt1	-	-	*Chelydra serpentina*	Rhen et al., 2007
Dmrt1	AF464141	-	*Calotes versicolor*	Sreenivasulu et al., 2002
Dmrt1	AF192560	-	*Alligator mississippiensis*	Smith et al., 1999a
Dmrt1	AF209095	Not Y-linked	*Oncorhynchus mykiss*	Marchand et al., 2000 Alfaqih et al., 2009
Dmrt1	AY157562	DRE 5	*Danio rerio*	Guo et al., 2004a Guo et al., 2005
Dmrt1	NM_001037949.1	-	*Takifugu rubripes*	Brunner et al., 2001
Dmrt1	AAN65377	-	*Xiphophorus maculatus*	Veith et al., 2003

Gene Symbol	NCBI Reference mRNA Sequence	Chromosome Localisation	Organism	References
Dmrt1	AY319416	-	*Odontesthes bonariensis*	Fernandino et al., 2006
Dmrt1	AF421347	-	*Monopterus albus*	Huang et al., 2002 Huang et al., 2005a
tDmrt1	AF203489	Not Y-linked	*Oreochromis niloticus*	Guan et al., 2000
tDMO	AF203490	-	*Oreochromis niloticus*	Guan et al., 2000
DMY/Dmrt1bY	AB071534	OLA Y	*Oryzias latipes*	Matsuda et al., 2002 Nanda et al., 2002
Dmrt1/Dmrt1a	-	OLA LG9	*Oryzias latipes*	Brunner et al., 2001 Nanda et al., 2002
DMRT2	NM_001130865.2	HSA 9p24.3	*Homo sapiens*	Raymond et al., 1999a Ottolenghi et al., 2000b
Dmrt2	NM_145831.3	MMU 19C1	*Mus musculus*	Kim et al., 2003
Dmrt2	NM_001192373	BSA 8q17	*Bos taurus*	Bratuś & Słota; 2009
Dmrt2	XM_003480526	SSC 1q21	*Sus scrofa domestica*	Bratuś & Słota; 2009
Dmrt2	ENSOANT00000013193[8]	OAN X5q	*Ornithorhynchus anatinus*	El-Mogharbel et al., 2007
Dmrt2	AY960292	-	*Gallus gallus*	Saúde et al., 2005
Dmrt2	AB264329	-	*Rana rugosa*	Matsushita et al., 2007
Dmrt2	AF209096	-	*Oncorhynchus mykiss*	Marchand et al., 2000
Dmrt2a	AF319992	OLA LG9	*Oryzias latipes*	Brunner et al., 2001
Dmrt2a	NM_001037946.1	-	*Takifugu rubripes*	Brunner et al., 2001
Dmrt2a	AAL83920	-	*Xiphophorus maculatus*	Kondo et al., 2002
Dmrt2a/terra	NM_130952	DRE 5	*Danio rerio*	Meng et al., 1999 Guo et al., 2004a
Dmrt2b	NM_001079976	DRE 6	*Danio rerio*	Zhou et al., 2008
DMRT3/DMRTA3	NM_021240.2	HSA 9p24.3	*Homo sapiens*	Ottolenghi et al., 2002
Dmrt3	NM_177360.3	MMU 19C1	*Mus musculus*	Kim et al., 2003
Dmrt3	XM_001788026	BSA 8q17	*Bos taurus*	Bratuś & Słota; 2009
Dmrt3	-	SSC 1q21	*Sus scrofa domestica*	Bratuś & Słota; 2009
Dmrt3	XM_001507779.2	OAN X5q	*Ornithorhynchus anatinus*	El-Mogharbel et al., 2007
Dmrt3	XP_427822.1	-	*Gallus gallus*	Smith et al., 2002
Dmrt3	AB264330	-	*Rana rugosa*	Matsushita et al., 2007
Dmrt3	AF319993	OLA LG9	*Oryzias latipes*	Brunner et al., 2001
Dmrt3	AY621083	DRE 5	*Danio rerio*	Guo et al., 2004a
Dmrt3	NM_001037945.1	-	*Takifugu rubripes*	Brunner et al., 2001
DMRT4/DMRTA1	NM_022160.2	HSA 9p21-22	*Homo sapiens*	Ottolenghi et al., 2002
Dmrt4/Dmrta1	NM_175647.3	MMU 4C4	*Mus musculus*	Kim et al., 2003
Dmrt4	AY648303	-	*Xenopus leavis*	Huang et al., 2005b
Dmrt4	AF209097	-	*Oncorhynchus mykiss*	Marchand et al., 2000
Dmrt4	-	OLA LG18	*Oryzias latipes*	Kondo et al., 2002
Dmrt4	AB201464.1	-	*Takifugu rubripes*	Yamaguchi et al., 2006
Dmrt4	CAF90474	-	*Xiphophorus maculatus*	Kondo et al., 2002
DMRT5/DMRTA2	NM_032110.2	HAS 1p32.3-33	*Homo sapiens*	Ottolenghi et al., 2002
Dmrt5/Dmrta2	NM_172296.2	MMU 4C7	*Mus musculus*	Kim et al., 2003

Gene Symbol	NCBI Reference mRNA Sequence	Chromosome Localisation	Organism	References
Dmrt5	AB264331	-	*Rana rugosa*	Matsushita et al., 2007
Dmrt5	AY618549	DRE 8	*Danio rerio*	Guo et al., 2004b
Dmrt5	AB201465.1	-	*Takifugu rubripes*	Yamaguchi et al., 2006
Dmrt5	DQ335470	-	*Xiphophorus maculatus*	Veith et al., 2006a
DMRT6/DMRTB1	NM_033067.1	HSA 1p32.2	*Homo sapiens*	Ottolenghi et al., 2002
Dmrt6/Dmrtb1	NM_019872.1	MMU 4C7	*Mus musculus*	Kim et al., 2003
DMRT7/DMRTC2	NM_001040283.1	HSA 19q13.2	*Homo sapiens*	Ottolenghi et al., 2002
Dmrt7/Dmrtc2	NM_027732.2	MMU 7A3	*Mus musculus*	Kim et al., 2003
Dmrt7/Dmrtc2	XM_218456	RNO 1q21	*Rattus norvegicus*	Veith et al., 2006b
Dmrt7	ENSOANT00000021 972[8]	-	*Ornithorhynchus anatinus*	Tsend-Ayush et al., 2009
DMRT8/DMRTC1	NM_033053.2	HSA Xq13.2	*Homo sapiens*	Ottolenghi et al., 2002
Dmrt8.1/Dmrtc1a	NM_001038616.2	MMU XD	*Mus musculus*	Veith et al., 2006b
Dmrt8.1/Dmrtc1a	NM_001025288	RNO X	*Rattus norvegicus*	Veith et al., 2006b
Dmrt8.2/Dmrtc1b	NM_001039116.2	MMU XD	*Mus musculus*	Veith et al., 2006b
Dmrt8.2/Dmrtc1b	XM_228580	RNO Xq13	*Rattus norvegicus*	Veith et al., 2006b
Dmrt8.3/Dmrtc1c1	NM_001142691.1	MMU XD	*Mus musculus*	Veith et al., 2006b
Dmrt8.3/Dmrtc1c1	NM_001014222	RNO Xq13	*Rattus norvegicus*	Veith et al., 2006b

*the ENSEMBL reference sequence (available at www.ensembl.org),
'-' cDNA sequences published neither in databases nor in given references/unknown chromosome localisation

Table 1. **DM-domain genes in representative vertebrates.** The presented nomenclature of DM domain genes is adopted from Volff (Volff et al., 2003a) or described in given references. The DM domain genes chromosomal localisations linked to sex chromosomes are indicated in grey fields.

The second DM domain gene in humans, *DMRT2*, was first identified by Raymond and co-workers, who mapped it to the same chromosomal band (HSA 9p24.3) as *DMRT1* (Raymond et al., 1999a). Both genes were shown to be deleted in the sex-reversing 9p monosomy, and therefore, *DMRT2* was also considered to be partially responsible for the XY sex-reversal phenotype in humans. Further studies, however, have provided evidence of *DMRT2* as a less likely sex-developmental candidate locus. First, it was mapped outside the deleted region in the newly refined 9p microdeletion in two XY sex-reversed females (Calvari et al., 2000). Second, its expression appeared to be widespread in adult human tissues (not restricted to testis) (Ottolenghi et al., 2000b). Third, DNA sequence analysis showed its high identity (100% in the DM domain) with the previously described DM domain gene in zebrafish, named *terra*, which was evidenced to be involved in somitogenesis but not sex development (Meng et al., 1999). Subsequent studies carried out in other vertebrates and based on both expression and functional analyses have indeed confirmed these preliminary presumptions (Tables 3 and 4).

Interestingly, further detailed screening of PAC/BAC clones overlapping the chromosomal region in humans associated with 46,XY gonadal dysgenesis and mapped to the tip of chromosome 9 (HSA 9p24.3) has revealed an additional (i.e., in addition to *DMRT1* and *DMRT2*) locus with the DM domain named *DMRT3* with a position proximal to *DMRT1*

and distal to *DMRT2* (Ottolenghi et al., 2000a). What is more, the newly described human cluster of DM domain genes, *DMRT1-DMRT3-DMRT2*, was later discovered to be a very conservative vertebrate locus. It was surprisingly found to be isolated from different fish species (i.e., medaka *O. latipes*, pufferfish *F. rubripes* (Brunner et al., 2001), zebrafish *D. rerio* (Guo et al., 2004a)) and from mice (Kim et al., 2003), rats (Guo et al., 2004a), platypus (El-Mogharbel et al., 2007), pigs and cattle. However, in these two last species, the order of *DMRT* genes was different (Bratuś & Słota, 2009).

It is now known that eight *DMRT* genes exist in human and mouse genomes (Ottolenghi et al., 2002; Kim et al., 2003; Veith et al., 2006b) (Table 1), which, compared to four and eleven DM domain loci previously isolated from invertebrates *D. melanogaster* and *C. elegans* respectively, is not surprising (reviewed by Volff and collaborators; Volff et al., 2003a). The subsequent expression and selected functional studies in numerous vertebrate species (Tables 3 and 4) have shown the variability in the expression profiles between both DM domain paralogs and homologs. Although the involvement of multiple DM domain genes in vertebrate sexual development was supported and might be considered a general phenomenon in developmental biology, it is obvious that *DMRT* genes also regulate the development of other organs during vertebrate embryogenesis (Tables 3 and 4). The recent data are discussed below in detail.

3. Sexual contra somatic embryo development: The involvement of DM domain genes

In order to determine the role of the genes in sexual development, both expression and functional studies have to be carried out. DM domain genes, as mentioned before, are molecular regulators of developmental processes that take place in the embryo. The embryo is, therefore, the main object used to study the function of *DMRT* genes. However, concerning humans, ethical issues arise. In this respect, performing studies in model organisms is often the only alternative. In the case of DM domain genes, extending investigations to all vertebrate phyla has brought new, interesting data about the evolution of this gene family.

Numerous DM domain genes were studied in different animal models employing various sex-determination strategies: genetic: (male or female heterogamety in XX/XY or ZZ/ZW systems, respectively), environmental (temperature, social factors) or a combination (Table 2). Different molecular biology methods were used to study the spatial and temporal expression of DM domain genes during embryogenesis. Both the mRNA and protein levels were measured either by very sensitive amplification methods (RT-PCR, quantitative RT-PCR) or less sensitive hybridisation techniques (Northern blot, Western-blot). In order to identify the cell type of the developing organ where the gene expression took place, the whole-mount *in situ* hybridisation (using gene-specific RNA probes) and/or immunohistochemistry methods (with specific antibodies) were applied to embryo sections. Since transcription factors, the proteins that regulate the expression of other genes by binding to the DNA sequence in their vicinity, are the final *DMRT* gene expression products, the chromatin immunoprecipitation (ChIP) method was employed to determine the upstream/downstream *DMRT* regulators in the embryo developmental pathways. What is more, both *DMRT* expression and ChIP techniques were supplemented by the next-generation technologies that currently provide tools for whole-genome investigations, such

as DNA microarrays (cDNA arrays and ChIP-chip, respectively). Moreover, functional studies, which provide the strongest evidence for gene-role determination, were carried out in different animal models (mostly in mice and in various fish species) and were based on artificial single-gene modifications like the loss of function mutation (e.g., knockout/knockdown of the gene) or the gain of function mutation (e.g., induced gene over-expression).

The function of *DMRT* genes in the developmental pathways of various vertebrate species is here broadly compared and summarised.

3.1 *DMRT1*, vertebrate sexual regulator

There is no doubt that among DM domain genes, *DMRT1* has been the most extensively investigated. A careful on-line search of the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/) provided the wide collection of data about *DMRT1* expression during vertebrate embryogenesis and in postnatal/adult animal tissues (Table 2).

So far, *DMRT1* appears to have a gonad-specific and sexually dimorphic expression profile during embryogenesis in all vertebrates tested (from mammals to fish). Besides this conservative status of *DMRT1* as the universal vertebrate sexual regulator (which might be considered a new phenomenon in animal developmental biology), several lines of evidence supported its functional variability during vertebrate gonad development. Is this more of a sex determination or a sex-differentiation locus? Is it involved only in male gonad formation, or does it also play a role in ovary development? The expression and functional studies undertaken in a wide range of vertebrate species have resolved some of the above questions.

In most cases, *DMRT1* is up-regulated either late during sex-determination or during the early testis-differentiation period. This subtle difference in its temporal expression during embryogenesis in various vertebrates makes its function vary significantly more among species.

Dmrt1 may be considered a switch sex-determining gene in reptiles employing a temperature-dependent sex-determining strategy. In separate studies of different reptilian species (i.e., crocodiles (*Alligator mississippiensis*) and turtles (*Trachemys scripta*, *Lepidochelys olivacea*, *Chelydra serpentine* (Table 2)), it has been shown that *Dmrt1* is the earliest genetic factor whose expression is temperature sensitive: The mRNA level of the gene was higher in embryos incubated in a male-promoting temperature than in embryos incubated in a female-promoting temperature. If the hypothesis that *Dmrt1* is more likely to be itself temperature sensitive and auto-regulatory than to be regulated by another unidentified sensitive-temperature genetic factor is supported, *Dmrt1* may primarily play a male-determining role (Zarkower, 2001). However, no functional studies have been carried out in this vertebrate phylum. That is not the case in birds, where both expression (Table 2) and functional analyses (Table 3) have confirmed the sex-determination status of avian *Dmrt1*. Sex is chromosomally based (ZZ males/ZW females) in birds, but sex determination had been a long-standing mystery. The bird homolog of the previously identified mammalian master-determining *Sry* (Sinclair et al., 1990; Koopman et al., 1991) has not been isolated from the avian genome. Thus, two hypotheses have been proposed

regarding the mechanism of sex determination in birds. The primary switch gene may be either a W-linked female dominant factor or a dosage-sensitive gene residing on the Z chromosome and triggering testis development. *Dmrt1*, which has been shown to be Z-linked in different bird species (Nanda et al., 2000; Shetty et al., 2002), is transcribed specifically during chick embryogenesis. Its expression becomes sexually dimorphic before the onset of sex differentiation: It is stronger in developing male than female gonads (Table 2). The elevated expression of *Dmrt1* from two Z chromosomes (unlike the mammalian X chromosome, there is no dosage compensation in birds) in the genital ridge at the time of sex determination may initiate testis differentiation, whereas one gene dosage is insufficient and lets ZW gonads follow a default female pathway. The *Dmrt1* Z dosage hypothesis for chicken sex determination was finally confirmed by the latest functional studies (Table 3), in which *Dmrt1* knockdown ZZ embryos successfully showed significant gonad feminisation (Smith et al., 2009). Although this spectacular finding closes the large gap in the bird sex-determination pathway, further studies of other avian species have to be undertaken in order to confirm/exclude the universal *Dmrt1* status as the bird sex-determining gene.

Phylum	Species	Sex-determination strategy	**Expression** (placement/molecular level/methods)		References
			Embryo	Postnatal/Adult Tissue	
Mammals	Human (*H. sapiens*)	GSD, XX females XY males Dominant Y		T/mRNA/DB	Raymond et al., 1998
			T/mRNA/ISH		Moniot et al., 2000
				T/mRNA/qRT-PCR	Cheng et al., 2006
	Mouse (*M. musculus*)		T+O/mRNA/ISH		Smith et al., 1999a
			T+O/mRNA/ ISH & RT-PCR	T/mRNA/RT-PCR	Raymond et al., 1999b
			T+O/mRNA/ISH	T/mRNA/NB & ISH	De Grandi et al., 2000
				T/protein/IHC	Raymond et al., 2000
				O/protein/IHC	Pask et al., 2003
			T+O/mRNA/ RT-PCR & qRT-PCR	T/mRNA/NB & RT-PCR & qRT-PCR	Lu et al., 2007
			T+O/protein/IHC	T/protein/WB	Lei et al., 2007
	Rat (*R. norvegicus*)		T/mRNA/RPA	T/mRNA/RPA	Chen & Heckert, 2001
	Pig (*S. scrofa*)			T+O+K/mRNA/ RT-PCR	Bratuś & Słota, 2009
	Cattle *(B. taurus*)			T+O+K+L+H+M+LU+S/mRNA/RT-PCR	Bratuś & Słota, 2009
	Tammar wallaby (*M. eugenii*)	GSD, Dominant Y	T+O/protein/IHC	T+O/protein/IHC	Pask et al., 2003
	Platypus (*O. anatinus*)	GSD, 5X+5Y males 2x5X females		T/mRNA/RT-PCR; T+O/protein/IHC	El-Mogharbel et al., 2007 Tsend-Ayush et al., 2009
Birds	Chicken (*G. gallus*)	GSD, ZZ males ZW females Dosage Z	T+O/mRNA/ISH		Smith et al., 1999a Raymond et al., 1999b
			T+O/mRNA/ISH	T/mRNA/NB & RT-PCR	Shan et al., 2000
			T+O/mRNA/ISH & qRT-PCR;		Smith et al., 2003

Phylum	Species	Sex-determination strategy	**Expression** (placement/molecular level/methods)		References
			Embryo	Postnatal/Adult Tissue	
			T+O/protein/IHC		
			T+O/mRNA/ RT-PCR & ISH	T+H/mRNA/RT-PCR; T/mRNA/NB	Zhao et al., 2007
	Emu (*D. novaehollandeae*)	GSD, Dosage Z?	Embryos of both sexes/mRNA/RT-PCR		Shetty et al., 2002
Reptiles	Alligator (*A. mississippiensis*)	TDS	T+O/mRNA/RT-PCR		Smith et al., 1999a
	Red-eared slider turtle (*T. scripta*)	TDS	T+O/mRNA/ISH & RT-PCR		Kettlewell et al., 2000
			T+O/mRNA/qRT-PCR		Murdock & Wibbels, 2003
	Sea turtle (*L. olivacea*)	TDS	T+O/mRNA/RT-PCR		Torres-Maldonado et al., 2002
	Snapping turtle (*C. serpentine*)	TDS	T+O/mRNA/qRT-PCR		Rhen et al., 2007
	Indian garden lizard (*C. versicolor*)	unknown	T+O/mRNA/ qRT-PCR & ISH	T/mRNA/RT-PCR	Sreenivasulu et al., 2002
Amphibians	Frog (*R. rugosa*)	GSD, XX females XY males	T/mRNA/RT-PCR	T/mRNA/RT-PCR	Shibata et al., 2002
			T/protein/IHC	T/mRNA/ISH; T/protein/IHC	Aoyama et al., 2003
	Clawed frog (*X. laevis*)	GSD, Dosage Z? Dominant W?	T+O/mRNA/RT-PCR	T/mRNA/NB & RT-PCR	Osawa et al., 2005
			T+O/mRNA/ISH & RT-PCR		Yoshimoto et al., 2006 Yoshimoto et al., 2008
Fish	Rainbow trout (*O. mykiss*)	GSD, XX females XY males	T+O/mRNA/NB & RT-PCR	T/mRNA/NB; T+O/mRNA/RT-PCR	Marchand et al., 2000
tDMRT1	Nile tilapia (*O. niloticus*)	GSD, XX females XY males		T/mRNA/NB	Guan et al., 2000
tDMO	Nile tilapia (*O. niloticus*)			O/mRNA/NB	
DMRT1a	Medaka (*O. latipes*)	GSD, XX females XY males Dominant Y	Undetectable/mRNA/RT-PCR	T/mRNA/RT-PCR	Brunner et al., 2001 Nanda et al., 2002
			Undetectable/mRNA/RT-PCR & ISH	T+O/mRNA/ISH	Winkler et al., 2004
			T/mRNA/RT-PCR	T/mRNA/RT-PCR	Kobayashi et al., 2004
DMY/DMRT1BY	Medaka (*O. latipes*)		Detectable in XY embryos/mRNA/RT-PCR	T/mRNA/RT-PCR	Nanda et al., 2002 Kobayashi et al., 2004
	Japanese pufferfish (*T. rubripes*)	Unknown	T/mRNA/RT-PCR & ISH	T+O/mRNA/RT-PCR	Yamaguchi et al., 2006
	Green spotted puffer (*T. nigroviridis*)	Unknown		T+O/mRNA/RT-PCR	Brunner et al., 2001
	Zebrafish (*D. rerio*)	Unknown		T+O/mRNA/RT-PCR & qRT-PCR & NB & ISH	Guo et al., 2005
	Platyfish (*X. maculatus*)	GSD, XX females,	Undetectable/mRNA/ISH	T/mRNA/RT-PCR & ISH	Veith et al., 2006a

Phylum	Species	Sex-determination strategy	**Expression** (placement/molecular level/methods)		References
			Embryo	Postnatal/Adult Tissue	
		XY males			
	Pejerrey, (*O. bonariensis*)	TDS		T/mRNA/RT-PCR	Fernandino et al., 2006
			T+O?mRNA/qRT-PCR		Fernandino et al., 2008
	Atlantic cod (*G. morhua L.*)			T+O/mRNA/ RT-PCR & qRT-PCR & ISH	Johnsen et al., 2010
	Rice field eel *(M. albus)*			T+O+B/mRNA/ RT-PCR & qRT-PCR; O+T/mRNA/NB	Huang et al., 2005a

Table 2. ***DMRT1* expression in vertebrates**. GSD-genetic sex determination, TDS-temperature dependence sex determination, T-testis/genital ridge in male embryo, O-ovary/genital ridge in female embryo, K-kidney, L-liver, H-heart, M-muscle, LU-lung, S-spleen, ISH-*in situ* hybridisation, RT-PCR-reverse transcription-polymerase chain reaction, qRT-PCR-quantitative RT-PCR, NB-Northern blot, DB-Dot blot, IHC-immunohistochemistry, WB- Western blot, RPA-RNase protection assay.

In fish, it is already known that *Dmrt1* is the unique male sex-determination locus, exclusively identified in a single fish species, medaka *O. latipes*. Medaka, unlike many other fish, uses a simple genetic mechanism similar to that found in mammals, with XX females and XY males. Surprisingly, two research groups simultaneously but independently found that the duplicated copy of previously isolated autosomal *Dmrt1/Dmrt1a* locus (Brunner et al., 2001) is located on the Y chromosome in its sex-determination region. This new paralog was named after the authors: *Dmrt1bY* (Nanda et al., 2002) or *DMY* (Matsuda et al., 2002). Its specific expression pattern during embryogenesis (it is transcribed early and exclusively in XY embryos) (Table 2) and the molecular analysis of XY *DMY* mutants that appeared to be male-to-female sex reversed (Matsuda et al., 2002) are consistent with its sex-determination function. Thus, medaka *Dmrt1bY/DMY* represents the unique non-mammalian vertebrate equivalent of *Sry*; however, it is not described in any other fish species, regardless of their relation to medaka (i.e., whether close or distant) (Kondo et al., 2003; Volff et al., 2003b; Veith et al., 2003).

What is, then, the role of *Dmrt1* in mammals that exhibit a genetic sex-determining mechanism (XX females/XY males) with the well-described Y-borne male-dominant locus of *Sry*? Intriguingly, the latest detailed studies have presented some functional diversity.

The data from humans, similar to that from chicken and medaka, are consistent with the hypothesis that *DMRT1* dosage is crucial for sex determination. Male-to-female sex reversal in XY individuals with monosomic deletion of 9p (bearing *DMRT1*) 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 report of Moniot and others (Moniot et al., 2000) showed co-expression of *SRY* and *DMRT1* in the genital ridge of the human male but not in the female embryo at the time when gonads appear morphologically undifferentiated. This male-specific expression of *DMRT1* in early gonadogenesis prior to sex differentiation suggests a partial (shared with *SRY*) role in

human sex determination. Unlike human homolog, murine *Dmrt1*, which has been extensively examined during embryogenesis (Table 2) and in genetically modified mouse models (Table 3), appeared to play an essential role in male gonad differentiation but not sex determination. Its early expression in the genital ridges of both sexes became XY-specific (up-regulated in developing male gonads) after the activation of the *Sry* gene (Smith et al., 1999a; De Grandi et al., 2000). Furthermore, male *Dmrt1* knockout mice were found to have postnatal affected testes but were not sex reversed (Raymond et al., 2000). Murine *Dmrt1*, however, through its expression in premeiotic germ cells and in Sertoli cells of both foetal and postnatal gonads, controls many aspects of testicular development, including differentiation, proliferation, migration and pluripotency of germ cells as well as proliferation and differentiation of Sertoli cells (Fahrioglu et al., 2007; Kim et al., 2007; Krentz et al., 2009).

Despite the well-evidenced redundant function of *Dmrt1* in ovary development due to fully fertile *Dmrt1*$^{-/-}$ XX mouse mutants (Raymond et al., 2000), the latest studies provide some unexpected data suggesting the involvement of mammalian *Dmrt1* in female gonad differentiation. In contrast to humans, both DMRT1 proteins (mouse, tammar wallaby) and *Dmrt1* transcripts (pig, cattle)—together with their expression in testes—were detected in adult ovaries (Table 2). What is more, the latest genome-wide studies have revealed that murine *Dmrt1* is a bi-functional transcriptional regulator that activates some genes and represses others. This not only occurs in juvenile testes, where *Dmrt1* acts differently depending on the testis cell line (Murphy et al., 2010). *Dmrt1* also can regulate the same gene target sex-specifically. *Stra8* (Stimulated by retinoic acid 8), the well-known meiotic inducer, is directly activated by *Dmrt1* in foetal ovary germ cells, which results in oogenesis initiation, whereas in adult testes, *Stra8* is transcriptionally repressed, showing *Dmrt1*-depandant control of spermatogenesis (Krentz et al., 2011). Although *Dmrt1*$^{-/-}$ mutant females were fertile (having reduced but enough functional ovarian follicles), the latest report of Krentz's group has finally demonstrated that *Dmrt1* does indeed function in the foetal ovary (Krentz et al., 2011).

In lower vertebrates, as in mammals, *Dmrt1* mRNA was also expressed in adult ovarian tissue of several fish species (Table 2). Moreover, in addition to the testis-specific *tDmrt1,* the other DM domain gene (*tDMO*) was isolated from one teleost fish, the tilapia (Guan et al., 2000). *tDMO* (tilapia DM domain gene in Ovary), the expression of which is limited to the ovary in adult animals, is the first-described female-specific DM domain gene in vertebrates. In contrast to the alternatively spliced male and female invertebrate *doublesex* (Burtis & Baker, 1989), *tDmrt1* and *tDMO* cDNAs appear to be encoded by two different genes that share little homology outside the DM domain.

However, more spectacular were functional studies carried out by Yoshimoto and co-workers (Yoshimoto et al., 2008; Yoshimoto et al., 2010), who isolated a W-linked *DM-W*. This is a paralog of *Dmrt1* in a single amphibian species, the African clawed frog *Xenopus leavis*, which has a ZZ/ZW-type sex-determining system. Both the *DM-W* transient expression in ZW tadpoles in the period of sex determination and the functional analysis of ZZ transgenic tadpoles carrying a *DM-W* expression vector and showing ovarian cavities and primary oocytes has suggested that *DM-W* is a likely sex (ovary)-determining locus in *X. leavis*, probably acting by antagonising *Dmrt1* (Yoshimoto et al., 2010).

Function	Gene	Species	References
Male sex determination	*Dmrt1*	*Gallus gallus*	Smith et al., 2003 Smith et al., 2009
	DMY/Dmrt1bY	*Oryzias latipes*	Matsuda et al., 2002
Male sex differentiation	*Dmrt1*	*Mus musculus*	Raymond et al., 2000 Boyer et al., 2002 Fahrioglu et al., 2007 Kim et al., 2007a Krentz et al., 2009 Matson et al., 2010 Matson et al., 2011
		Rattus norvegicus	Lei et al., 2009
	Dmrt7	*Mus musculus*	Kawamata & Nishimori, 2006 Kim et al., 2007b
	Dmrt4	*Mus musculus*	Balciuniene et al., 2006
Female sex determination	*DM-W*	*Xenopus laevis*	Yoshimoto et al., 2008 Yoshimoto et al., 2010
Female sex differentiation	*Dmrt1*	*Mus musculus*	Krentz et al, 2011
	Dmrt4	*Mus musculus*	Balciuniene et al., 2006
Muscle development	*Dmrt2*	*Mus musculus*	Seo et al., 2006 Seo, 2007 Sato et al., 2010 Lourenço et al., 2010
	terra/Dmrt2a	*Danio rerio*	Meng et al., 1999 Saúde et al., 2005
	Dmrt2b	*Danio rerio*	Liu et al., 2009
Neurogenesis	*Dmrt4*	*Xenopus laevis*	Huang et al., 2005b

Table 3. **Functional studies of DM domain genes in vertebrates**.

Summarising the presented data, the vertebrate DM domain gene *Dmrt1* and its close paralogs act as primary-sex determining genes in different vertebrate phyla, including fish (*DMY/Dmrt1bY*), amphibians (*DM-W*) and birds (Z-linked *Dmrt1*), each with an independently evolved chromosomal sex-determination mechanism. Unlike sex chromosome-linked *Dmrt1* orthologs, autosomal *Dmrt1* genes appear as critical sex-differentiating (but not sex-determining) factors acting in developing embryonic/postnatal gonads in mammals (mouse), amphibians (frog *Rana rugosa*) and fish (medaka, Nile tilapia).

In species not having sex chromosomes with temperature-dependant sex-determination mechanisms (some reptiles), *Dmrt1* is a likely genetic factor that may play a primary sex-determination role.

From an evolutionary point of view, *Dmrt1* homologs are thought to be frequently recruited or retained to determine/differentiate sex as new sex-determination mechanisms arise.

Despite the wide knowledge about *Dmrt1* as the vertebrate sex-developmental locus, new studies, especially based on recently available high-throughput genome-wide technologies, are being performed in order to better understand its transcriptional regulation in testis/ovary differentiation pathways. Still, little is known about the *Dmrt1* targets or the manner in which their expression is regulated. What is more, the newest intriguing data about the *DMRT1* association with the testicular germ cell tumour (TGCT) in humans also requires further explanation (Kanetsky, et al., 2011; Turnbull et al., 2011).

3.2 DM domain genes, not just a sex issue

It is now well known that besides *Dmrt1*, seven other DM domain genes exist in the vertebrate genome (Table 1) (however, the numbers vary across species). Although they have not been studied as intensively as *Dmrt1*, recent findings provide a great deal of data about their embryonic expression pattern in different vertebrate clades, including mammals (mouse), birds (chicken), amphibians (frogs *R. rugosa*, *X. leavis*) and broadly investigated fish (medaka, zebrafish, platyfish, Japanese pufferfish). Following the extensive database search (as was done for *Dmrt1*), the newest knowledge about *DMRT* expression in both embryos and adult tissues in a variety of vertebrate species is summarised in Table 4.

A number of general statements can be deduced from this table. In addition to *Dmrt1*, most *Dmrt* genes are expressed in developing gonads during early embryogenesis, and in many cases, their expression is subsequently maintained at higher levels in male than in female gonads. However, in contrast to *Dmrt1*, many *Dmrt* genes are activated in other developing tissues/organs, either before or after the onset of their expression in gonads. This suggests that they may control a broader range of developmental processes. This non-gonad-restricted embryonic expression pattern was observed for *Dmrt2*, *Dmrt3*, *Dmrt4*, *Dmrt5*, *Dmrt6* and *Dmrt8.1*. In most species, *Dmrt* genes have been detected in mesodermally derived somites (mouse, chick and fish *terra*/*Dmrt2a* and chick *Dmrt3*), ectodermally derived olfactory placodes (mouse and chick *Dmrt3*; *Xenopus*, platyfish and medaka *Dmrt4*; and platyfish *Dmrt5*) and neuroectodermally derived developing brain (*Dmrt3*, *Dmrt4*, *Dmrt5* and *Dmrt6* in mouse, chicken, *Xenopus* and fish). It is important to emphasise that the expression of some *Dmrt* genes has not been carefully studied besides forming gonads, and therefore, their activation in other tissues may have been overlooked. For example, most murine *Dmrt* genes were analysed in a variety of organs but only at one developmental stage (E 14.5), and subsequent detailed investigations were carried out only in dissected embryonic gonads (Kim at al., 2003). Similarly, the data from the embryonic expression of some *Dmrt* genes in frog *Rana rugosa* were based on cDNA preparations from either whole embryos or gonads of tadpoles (Matsushita et al., 2007). Moreover, the choice of method is also crucial. It was often noticed that transcripts detectable by more sensitive RT-PCR are not visible in embryo sections following the less sensitive *in situ* hybridisation.

Gene	Organism	Expression in embryos	Expression in adult tissues	References
DMRT2	*H. sapiens*	embryos aged 4-7 weeks of both sexes[1]	K, SM, Th, L, I, **T**	Ottolenghi et al., 2000a Ottolenghi et al., 2000b Calvari et al., 2000
Dmrt2	*M. musculus*	*at E9.5* PSM, somites *at E14.5* B, **T**, H, **O**, K, BL, K, L, S, Li	**T**[2]	Meng et al., 1999 Kim et al., 2003
	S. scrofa	-	SM, B, K, **T**, **O**, Sp	Bratuś & Słota, 2009
	B. taurus	-	SM, K, **T**	Bratuś & Słota, 2009
	O. anatinus	-	K, **T**, **O**,	El-Mogharbel et al., 2007 Tsend-Ayush et al., 2009
	G. gallus	PSM, somites[3]	-	Saúde et al., 2005
	R. rugosa	**T, O**[4]	K, **T**, B	Matsushita et al., 2007
	O. latipes	*since day 2*, somites, PSM, *day 4*, somites, B	**T**, **O**, G	Brunner et al., 2001 Winkler et al., 2004
	T. rubripes	-	**T, O,** G, I, E, M	Yamaguchi et al., 2006
	X. maculatus	*since day 3,* somites, head	G	Veith et al., 2006a
terra/Dmrt2a	*D. rerio*	somites, PSM	M, **T**, O, B	Meng et al., 1999
Dmrt2b	*D. rerio*	branchial arches	M, Li, **O, T,** B	Zhou et al., 2008
DMRT3/DMRTA3	*H. sapiens*	-	**T**, B, L, SM	Ottolenghi et al., 2000a Ottolenghi et al., 2002
Dmrt3	*M. musculus*	*at E9.5* forebrain, nasal placodes *at E14.5* B, L, S, **T**, K, I	not expressed in T	Smith et al., 2002 Kim et al., 2003
	S. scrofa	-	**T**	Bratuś & Słota, 2009
	B. taurus	-	**T**	Bratuś & Słota, 2009
	O. anatinus	-	**T**	El-Mogharbel et al., 2007
	G. gallus	*since E1* PSM, somites, at *E2.1* telencephalon, olfactory placodes at E7.5 Müllerian duct	-	Smith et al., 2002
	R. rugosa	**T,O**	B, **T**	Matsushita et al., 2007
	O. latipes	*since day 3*, hindbrain, neural tube	**T**	Brunner et al., 2001 Winkler et al., 2004
	D. rerio	olfactory placodes, neural tube	**T**, **O**	Li et al., 2008
	T. rubripes	*at 115 days after hatching* **T**	**T, O**, G, B, Li, M,	Yamaguchi et al., 2006
DMRT4/DMRTA1	*H. sapiens*	-	Li, K, P, Pr, L, **T, O**	Ottolenghi et al., 2002
Dmrt4	*M. musculus*	*at E14.5* B, H, **O**, **T**, BL, K, I, L, S	**O,T,** PG, Li, H, K, Sp, Th, L, I	Kim et al., 2003 Balciuniene et al., 2006
	X. laevis	*since stage 17,* olfactory placodes, forebrain, telencephalon	-	Huang et al., 2005b
	O. latipes	*since day 1*, olfactory placodes, telencephalon	**T**, K, G, **O**, E, B	Kondo et al., 2002 Winkler et al., 2004
	T. rubripes	-	**T, O,** Sp	Yamaguchi et al., 2006

	X. maculatus	*since day 3,* olfactory placodes; *day 5*: olfactory placodes, branchial arches, B	G	Veith et al., 2006a
DMRT5/DMRTA2 *Dmrt5*	*H. sapiens*	-	**T**	Ottolenghi et al., 2002
	M. musculus	*at E13.5* B *at E14.5* B, **O**, K, H, L, S, **T**	**T**	Kim et al., 2003
	R. rugosa	**T,O**	B, H, **T**, **O**, P, K	Matsushita et al., 2007
	D. rerio	B	B, **T, O**	Guo et al., 2004b
	T. rubripes	-	Sp, B	Yamaguchi et al., 2006
	X. maculatus	*since day 3,* olfactory placodes; B, lenses, *day 5*: olfactory epithelium, B	B, E	Veith et al., 2006a
DMRT6/DMRTB1 *Dmrt6*	*H. sapiens*	-	**T**, P, **O**	Ottolenghi et al., 2002
	M. musculus	*at E14.5* B	**T**	Kim et al., 2003
DMRT7/DMRTC2 *Dmrt7*	*H. sapiens*	-	**T**, P	Ottolenghi et al., 2002
	M. musculus	*at E14.5* **O, T**	**T**	Kim et al., 2003 Kawamata & Nishimori, 2006 Kawamata et al., 2007
	O. anatinus	-	**T**	Tsend-Ayush et al., 2009
DMRT8/DMRTC1	*H. sapiens*	-	**T**, **O**, K, P, B, L	Ottolenghi et al., 2002
Dmrt8.1	*M. musculus*	*at E13.5* S, Me, I, **O, T,** L, K, H, head, neural tube	**T**	Veith et al., 2006b
Dmrt8.2	*M. musculus*	*at E13.5* **T, O**	**T**	Veith et al., 2006b

[1]human *DMRT* genes (with the exception of *DMRT2*) were not investigated in embryos
[2]the expression of murine *DMRT* genes in adult animals was tested only in male gonads (with the exception of *DMRT4*, *DMRT7* and *DMRT8*)
[3]chick *DMRT2* was detected in 2-somite and 14-somite stages of embryo development as well as in the node from stage 4 Hamburger and Hamilton (4HH) to stage 7HH
[4]in frog *Rana rugosa*, the expression of *DMRT2*, *-3* and *-5* was investigated in whole embryos at stages 16, 21, 23 and in the gonad/mesonephros complex of tadpoles at stages I, III, V.

Table 4. **Spatial and temporal expression of *DMRT2-3-4-5-6-7-8* genes during embryogenesis and in adult animals across different vertebrate species.** The order of the indicated tissues in the row correlates with the decreasing level of the detected expression (e.g., the murine *DMRT7* at the E14.5 was enriched in ovaries). B-brain, BL-bladder, E-embryonic day, E-eye, G-gills, H-heart, I-intestine, K-kidney, L-lung, Li-liver, M-muscle, Me-mesonephros, O-ovary, P-pancreas, PG-preputial gland, Pr-prostate, T-testis, PSM-Presomitic mesoderm, S-stomach, SM-skeletal muscle, Sp-spleen, Th-thymus,'-' not reported.

However, based on available data, further observations can be made. While the expression patterns for various *Dmrt* genes have appeared to be conserved across species, there are also some clear differences. For instance, the specific for *Dmrt4* expression profile in nasal placode and in telencephalon in *Xenopus*, medaka and platyfish appears to be *Dmrt3* characteristic in mouse and chicken. What is more, chick *Dmrt3* is additionally expressed in presomitic mesoderm, which is not true for its mouse and fish orthologs but typical for *Dmrt2* is mouse, zebrafish, platyfish and medaka. Additionally, *Dmrt1*, which has been

found to be exclusively expressed in developing and adult gonads of all vertebrate phyla, surprisingly appears to be expressed in extragonadal adult tissues in cattle (heart, spleen, skeletal muscle, kidney, lung, liver) and in pig (kidney) (Bratuś & Słota, 2009; Table 2). The bovine *Dmrt1* widespread tissue-expression profile closely resembles the transcription patterns described for *DMRT2*, *DMRT4* and *DMRT8* in adult human tissues (Table 4).

The above observations indicate that the expression patterns and presumably the function of some vertebrate members of the DM-domain gene family may have shifted during evolution (Hong et al., 2007).

It is obvious, however, that in addition to *Dmrt1*, some other DM domain genes are involved in sexual development. This statement was already suggested after the observation of a relatively mild *Dmrt1* mutant phenotype in mice (Raymond et al., 2000). No defects outside the gonads were observed in the *Dmrt1*$^{-/-}$ males, while *Dmrt1*$^{-/-}$ females were not affected. The lack of *Dmrt1*, thus, might have been compensated for by the activation of other DM domain genes during sexual differentiation. Mouse *Dmrt3*, *Dmrt5* and *Dmrt7* exhibit sex-specific expression in the early embryonic gonads (their expression becomes enriched either in developing testes (*Dmrt3*) or in developing ovaries (*Dmrt5*, *Dmrt7*) (Kim et al., 2003). Unlike *Dmrt3* and *Dmrt5*, but similar to *Dmrt1*, *Dmrt7* expression is restricted only to embryonic mouse gonads of both sexes and becomes postnatally testis specific. Although the early XX-enriched expression of *Dmrt7* makes this gene a candidate for a role in early ovary differentiation, further functional studies have shown that it is essential for male fertility (Kawamata & Nishimori, 2006; Kim at al., 2007b; Table 3). While *Dmrt7*-deficient female mice were fertile, adult null males were infertile due to the affected functioning of testicular germ cells. It has been found that the lack of *Dmrt7* in mice is associated with an arrest of spermatogenesis at the late pachyten stage and with abnormal sex chromatin modifications normally required for male meiotic progression (Kim at al., 2007b).

Like *Dmrt7*, another DM domain gene, *Dmrt8* seems to be mammalian specific (so far not described in other vertebrates) and exclusively expressed in the embryonic gonads of both sexes as well as in the testes of adult mice (Veith et al., 2006b). However, unlike *Dmrt7*, its function as a sex regulator is now highly speculated because of at least three reasons: 1) It is widely expressed in human adult tissues including brain, lung, kidney, pancreas and gonads, 2) One of its copy found in mice, *Dmrt8.1*, is expressed in multiple embryonic organs in a non-sex-specific manner, and 3) No functional studies have yet been carried out in order to determine its role in mammalian development.

Conversely, functional studies of another murine *Dmrt* gene, *Dmrt4*, have revealed its involvement in some aspects of sexual development (Balciuniene et al., 2006). Despite its widespread expression in both embryos and adults, *Dmrt4* mutant mice appear to be viable and fertile. However, two potential mutant phenotypes have been observed: 1) *Dmrt4*-deficient females have elevated numbers of polyovular follicles due to affected folliculogenesis, and 2) 25% of mutant males attempt to copulate with other males, suggesting a possible behavioural abnormality. This potential involvement of *Dmrt4* in proper ovary development and male sexual behaviour has not been found in previous functional studies carried out in frog *Xenopus*, suggesting that *Dmrt4* orthologs are not functionally conserved (Huang et al., 2005b). The effects of *Dmrt4* depletion in frog embryos have been shown to be consistent with its early embryonic expression pattern (Table 4). The

Dmrt4-deficient embryos showed specific disruption of the expression of known neuronal differentiation factor (Xebf2) in the olfactory placode. Later, during embryogenesis, mutants exhibited impaired neurogenesis in the olfactory epithelium. Moreover, the forced expression of *Dmrt4* was sufficient to activate neurogenic markers in cultured *Xenopus* explants. Therefore, it was proposed that *Xenopus Dmrt4* is a key regulator in neurogenesis but not in gonad development. Moreover, the maintained activity of some neuronal gene markers in the *Dmrt4* mutant nasal placode may suggest the compensatory activity of other DM domain genes, such as *Dmrt3* and *Dmrt5*.

Similarly, *Dmrt6* and *Dmrt2* have also been shown to be less likely sexual regulators. In contrast to the poorly investigated *Dmrt6*, the expression of which was found to be restricted to the developing brain in mouse embryos (Kim et al., 2003), *Dmrt2* has been extensively studied during vertebrate embryogenesis as well as in genetically modified model organisms (Tables 3 and 4). *Dmrt2* shows a conserved expression pattern during embryogenesis. *Dmrt2* is expressed primarily in the presomitic mesoderm and newly formed somites in various vertebrate clades, including mammals (mouse), birds (chicken) and fish (medaka, platyfish and zebrafish) (Table 4). This suggests its involvement in muscle development across species. The detailed functional analyses, however, performed only in mouse and zebrafish, have indeed confirmed this hypothesis, but they have also revealed that type of developmental processes regulated by *Dmrt2* can differ in these two organisms. In zebrafish, overexpression of *terra/Dmrt2a* (homolog of human and mouse *Dmrt2*) induced rapid apoptosis in the somitic mesoderm both *in vitro* and *in vivo*, suggesting that the *terra* activity needs to be strictly regulated for proper mesoderm development (Meng et al., 1999). Moreover, the depletion of *terra* activity in zebrafish embryos has revealed two important roles of this DM domain gene: 1) It is involved in the active mechanism responsible for the left-right asymmetry formation, fundamental to vertebrate body-plan creation, and 2) It is responsible for proper bilateral synchronisation of the segmentation clock in the mesoderm, essential for the normal development of bilateral structures such as skeletal muscles (Saúde et al., 2005). What is more, it was recently reported that due to a genome duplication event, zebrafish *terra/Dmrt2a* has a paralog named *Dmrt2b* (Zhou et al., 2008). Contrary to *terra/Dmrt2a*, which is present in all vertebrates, *Dmrt2b* duplication exists only in the fish genome. *Dmrt2b*, like *terra/Dmrt2a*, also showed a left-right asymmetry establishment function in zebrafish embryos. However, unlike its paralog, it regulates other aspects of somite differentiation affecting slow muscle development (Liu et al., 2009). Surprisingly, neither the regulation of left-right patterning in the mesoderm nor the involvement in symmetric somite formation has been observed for murine *Dmrt2* (Lourenço et al., 2010). Instead, mouse embryos lacking the *Dmrt2* function showed early somite patterning defects, perturbed somite maturation, abnormal skeletal muscle in myotome and affected onset of myogenesis (Seo et al., 2006; Sato et al., 2010). Thus, murine *Dmrt2* and both zebrafish paralogs, *terra/Dmrt2a* and *Dmrt2b*, appear to be *Dmrt* family members with a well-evidenced role in vertebrate muscle development and not sex determination/differentiation.

4. Conclusion

Summarising the presented story about DM domain genes in vertebrates, it is a privilege for me to adopt one conclusion that has been proposed by professor Zarkower in his excellent

review paper about sexual development. "Conservation amidst diversity?" Ten years of further extensive investigations have brought the wide, fascinating knowledge about the DM domain gene family that perfectly reflects the cited conclusion. However, there has been one minor change: The question mark is not needed anymore.

5. Acknowledgment

I would like to thank Stefan for his patience and constant support.

6. References

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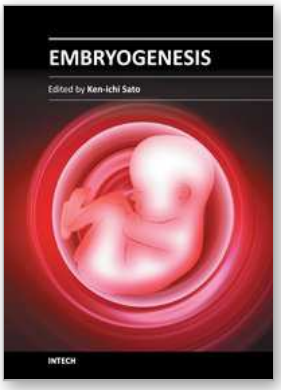

Embryogenesis

Edited by Dr. Ken-Ichi Sato

ISBN 978-953-51-0466-7
Hard cover, 652 pages
Publisher InTech
Published online 20, April, 2012
Published in print edition April, 2012

The book "Embryogenesis" is a compilation of cutting edge views of current trends in modern developmental biology, focusing on gametogenesis, fertilization, early and/or late embryogenesis in animals, plants, and some other small organisms. Each of 27 chapters contributed from the authorships of world-wide 20 countries provides an introduction as well as an in-depth review to classical as well as contemporary problems that challenge to understand how living organisms are born, grow, and reproduce at the levels from molecule and cell to individual.

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