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Review

The function of *Dmrt* genes in vertebrate development: It is not just about sex

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Abstract

The *Dmrt* genes encode a large family of transcription factors whose function in sexual development has been well studied in invertebrates and vertebrates. Their expression pattern is not restricted to the developing gonads, indicating that *Dmrt* genes might regulate other developmental processes. Here we review the expression pattern of several members of this family across species and summarize recent findings on the function of a subset of these genes in non-gonadal tissues.

Keywords: DM domain; Dmrt; Gonads; Sex determination; Somites; Laterality; Nasal placode; Olfactory system

The *Dmrt* gene family

Genes related to the *Drosophila melanogaster doublesex* (*dsx*) and *Caenorhabditis elegans mab-3* genes encode transcription factors conserved during evolution (reviewed in Zarkower, 2001; Volff et al., 2003). They constitute the *Dmrt* (*d*oublesex and *m*ab-3-*r*elated *t*ranscription factor) gene family, a class of molecules characterized by a DNA-binding motif known as the DM domain (Raymond et al., 1998). The DM domain is an unusual cysteine-rich DNA binding motif. It has a highly intertwined structure that chelates two atoms of zinc, and binds to the minor groove of the DNA (Zhu et al., 2000). Outside the DM domain there is little sequence similarity between *dsx* and *mab-3*. These genes play important roles in sex determination in flies and worms, respectively. In flies the sex-specific isoforms of *dsx* (dsx^M and dsx^F) regulate most aspects of somatic sexual dimorphism. The expression of the Drosphila yolk protein genes, which are synthesized in large quantity in adult females is directly regulated by dsx (reviewed in Bownes, 1994). Male *C. elegans* mutants for mab-3 synthesize yolk proteins and show defects in male genital development (Shen and Hodgkin, 1988), a phenotype similar to male flies lacking dsx^M function. The male isoform of the fly *dsx* gene, dsx^M (but not dsx^F) can partially compensate for the loss of *mab-3* in *C. elegans* (Raymond et al., 1998) indicating that their function is interchangeable and that sex determination in invertebrates relies upon conserved mechanisms.

With the discovery of *Dmrt* homologues in most vertebrates it has been tempting to speculate that the conserved function in the control of sex determination might extend to other organisms in the form of a universal mechanism of sex determination prevailing in all animal phyla (reviewed in Zarkower, 2001; Volff et al., 2003). Consistent with this view most *Dmrt* genes isolated in vertebrates are expressed in the developing gonads. As a result, vertebrates DM family members have been primarily studied in the context of sexual development, where they appear to be more directly involved in sex differentiation rather than sex determination. However the way in which they mediate their activity is largely unexplored and possible downstream targets of these factors have yet to be identified in the context of the developing vertebrate gonads.

Recent findings indicate that members of this gene family are also expressed in other tissues besides the gonads suggesting they may control a broader range of developmental processes. Among the eight *Dmrt* genes currently known in vertebrates, four of them have been recently studied for their potential role in non-gonadal tissues and here we review these findings.

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Developmental expression of *Dmrt* genes

The developmental expression of *Dmrt* genes in the gonad has been extensively studied in several species and has been discussed in a number of recent reviews (Hodgkin et al., 2002; Smith and Sinclair, 2004; Matsuda, 2005). Table 1 summarizes the expression pattern of these genes during embryonic development in a number of species. A couple of general tendencies can be deduced from this table. First, *Dmrt* family members show conserved expression early on in the indifferent gonad and in most cases they are subsequently maintained at higher levels in male as opposed to female gonads. Second, following their initial expression in the gonads a subset of these genes show differential expression in a limited number of non-gonadal tissues and organs, and this is the case for *Dmrt2*, *Dmrt3*, *Dmrt4*, *Dmrt5* and *Dmrt6*. It is also important to emphasize that the expression of some *Dmrt* genes has not been carefully analyzed in tissues not directly involved with sex determination (Table 1), raising the possibility that in some instances their expression may have been overlooked.

In the mouse *Dmrt1* is first detected at E9.5 in the bipotential gonad of both sexes. At E12.5 *Dmrt1* transcripts are localized to the Sertoli cells as well as the germ cells of the testis. By day E15.5 *Dmrt1* is only weakly expressed in the ovary but persist strongly in the testis (Raymond et al., 1999a). In birds, *Dmrt1* is located on the Z chromosome (Nanda et al., 2000) and is therefore present at higher levels in male embryos (ZZ) as compared to female embryos (ZW) (Reviewed in Smith and Sinclair, 2004). While *Dmrt1* is restricted to the developing gonad in mouse, medaka and platyfish, the expression of *Dmrt1* outside the gonad has not been analyzed in other species. Similarly, *Dmrt7* and *Dmrt8* are detected in the ovary and/or the testis and their expression in non-gonadal tissues has not been reported. *Dmrt7* and *Dmrt8* are found in mammals but not in other vertebrate species suggesting that they might be two mammalian-specific *Dmrt* genes. *Dmrt8* is also atypical in this gene family as it lacks a DM domain at least in mouse and possibly in humans (Veith et al., 2006b).

Among the eight vertebrate *Dmrt* genes identified so far, five of them have been described in other tissues beside the gonads (Table 1). In most species *Dmrt* genes have been detected in tissues such as the central nervous system (*Dmrt3*, *Dmrt4, Dmrt5* and *Dmrt6* in mouse, chick, Xenopus or fish), nasal placodes (mouse and chick *Dmrt3*, Xenopus and platyfish *Dmrt4*, and platyfish *Dmrt5*) or somites (mouse, chick and fish *Terra/Dmrt2*, and chick *Dmrt3*). Fig. 1 illustrates the differential expression of some of these genes in non-gonadal tissues in medaka (*Dmrt3*, Fig. 1A and *Dmrt4*, Fig. 1B), platyfish (*Dmrt4*, Fig. 1C and *Dmrt5*, Fig. 1D), Xenopus (*Dmrt4*, Figs. 1E–G), chick (*Terra/Dmrt2*, Figs. 1H–J) and mouse (*Dmrt3*, Fig. 1K) embryos.

While there are conserved patterns of expression for the various *Dmrt* genes across species, there are also some clear differences. For example, in Xenopus, medaka and platyfish *Dmrt4* is strongly expressed in the nasal placode and the telencephalon (Huang et al., 2005b; Winkler et al., 2004; Veith et al., 2006a). However, in both mouse and chick, it is *Dmrt3* that is predominantly expressed in these tissues (Smith et al., 2002), while mouse *Dmrt4* appears to be more broadly expressed (Balciuniene et al., 2006). The otic placode and gall bladder expression of *Dmrt4* appears to be a unique feature of medaka (Winkler et al., 2004) and Xenopus (Huang et al., 2005b) embryos, respectively, as it has not been reported in other species. Additionally, while *Dmrt3* genes have additional expression domains in the neural tube of mouse, chick and medaka (Smith et al., 2002; Winkler et al., 2004), the chick *Dmrt3* gene is also expressed in the presomitic mesoderm (Smith et al., 2002). In contrast, in Medaka the only *Dmrt* gene detected in the presomitic mesoderm is *Terra/Dmrt2* (Winkler et al., 2004), similar to its zebrafish and mouse orthologs (Meng et al., 1999; Seo et al., 2006).

These divergences in the expression of *Dmrt* genes across species indicate that the expression patterns and presumably the function of some members of this gene family may have shifted during evolution.

The function of the *Dmrt* gene family in embryogenesis

As the name of the founding member of this gene family, doublesex, indicates these genes have been primarily implicated in sexual development. Several mammalian DM domain genes in addition to *Dmrt1* exhibit sex-specific expression in the early embryonic gonad (Kim et al., 2003), suggesting that the involvement of multiple DM domain genes in sexual development might be a general phenomenon. The first evidence that DM family members may have a role in other organs besides the gonads came from the identification of a zebrafish gene known as *Terra* (Meng et al., 1999), homologue of human and murine *Dmrt2*. *Terra* is primarily expressed in the presomitic mesoderm and the developing somites suggesting that not all DM domain proteins are strictly involved in sexual development and raising the possibility that they may also regulate the development of other organs (Meng et al., 1999).

Among the eight DM domain genes known in vertebrates, only four have been the object of functional analysis in fish, mouse or Xenopus, namely *Dmrt1*, *Dmrt2*, *Dmrt4* and *Dmrt7*. Table 2 summarizes some of these loss and gain of function studies and the resulting phenotypes.

Dmrt1 is required for male gonad differentiation

In humans DMRT1 is located on the short arm of chromosome 9 and deletion of this region (9p) is a cause of 46,XY gonadal dysgenesis, a condition that predisposes to gonadoblastoma and in some cases causes sex reversal of the XY embryonic gonad into ovarian tissue (reviewed in Ottolenghi and McElreavey, 2000). For this reason, DMRT1 was thought to have an important function in sex determination. However, so far no point mutations have been mapped to DMRT1 in patients with sex reversal (Raymond et al., 1999b). Because two other *Dmrt* genes are also found in this region of chromosome 9 (DMRT2 and DMRT3) it has been proposed that sex reversal in these patients is due to the combined loss of two or more DMRT genes (Raymond et al., 1999b). A study looking

at a submicroscopic deletion of the 9p region associated with sex reversal indicated that DMRT1 and DMRT2 fall outside the region deleted in chromosome 9 (Calvari et al., 2000), arguing against the possibility that 9p sex reversal is the result of the combined haploinsufficiency of DMRT1 and DMRT2.

In the mouse, *Dmrt1* is detected in the bipotential gonad, however, early events in testis differentiation occur normally in the knockout male mice, which have only defects in postnatal testis differentiation. The Sertoli cells of mutant animals overproliferate and fail to adopt a differentiated morphology. The germ cells of embryos lacking *Dmrt1* function fail to undergo radial migration, to enter meiosis and die prematurely. Because *Dmrt1* is expressed in both Sertoli cells and germ cells, it is unclear whether the loss of germ cells in these mutants is a cell autonomous defect or an indirect consequence of defective Sertoli cells. To address this question, Kim et al. (2007b) have conditionally inactivated *Dmrt1* in the Sertoli cells and germ cell lineages using cell-type-specific Cre transgenes. This study indicates that *Dmrt1* is required in germ cells for radial migration and survival of gonocytes. In Sertoli cells *Dmrt1* is required autonomously for their proper differentiation, and non-autonomously for germ cells survival after the second postnatal week (Kim et al., 2007b). Altogether, these studies indicate that *Dmrt1* does not play a major early role in testis determination in mammals, but rather appears to be involved in multiple aspects of the male gonad differentiation.

A recent study has addressed the role of *Dmrt1* during the process of cell differentiation using NEC8 human embryonic carcinoma cell line (Koji et al., 2006). Phorbol ester-induced differentiation of NEC8 cells was found to be associated with an upregulation of *Dmrt1* possibly through the PKC/MAPK signaling pathway. Conversely, siRNA-knockdown of *Dmrt1* resulted in dramatic changes in cell morphology suggesting a possible role of *Dmrt1* as a differentiation switch (Koji et al., 2006). However, it is unclear at this time how these findings relate to male gonad differentiation.

The function of *Dmrt1* in sex determination has been also analyzed in species in which sex determination is influenced by environmental factors such as temperature changes. There is a very good correlation between the temperature-dependent sex determination of turtles, alligators and salamander, and increased *Dmrt1* expression at temperatures that promote male development (Smith et al., 1999; Kettlewell et al., 2000; Sakata et al., 2006). Nevertheless, the mechanisms regulating this process remain largely unknown (reviewed in Yao and Capel, 2005). In medaka, two *Dmrt1* genes are present, presumably as result of a recent duplication event of the autosomal *Dmrt1* genomic region. This additional copy, known as *Dmy*, maps to the sex-determining region of the Y chromosome (Nanda et al., 2002). This gene is necessary for male development (Matsuda et al., 2002), and unlike its mammalian homolog medaka *Dmy* appears to play a direct role in sex determination more similar to *Sry* function in mammals (Masuda et al., 2002). Interestingly, this gene is not present in closely related fish species (Kondo et al., 2003) and is not a primary sex determinant in zebrafish, where *Dmrt1* is detected in developing germ cells of both testis and ovary (Guo et al., 2005).

Altogether, these observations suggest that *Dmrt1* may not have a universal role as a sex-determining factor in vertebrates. A number of excellent reviews have more extensively discussed this aspect of *Dmrt1* function (Hodgkin, 2002; Koopman and Loffler, 2003; Smith and Sinclair, 2004; Schartl, 2004; Matsuda, 2005; Yao and Capel, 2005).

Dmrt2 is involved in establishing left–right asymmetry and regulates muscle development

In the zebrafish, *Terra* (*Dmrt2*) is transiently expressed in the presomitic mesoderm and in the newly formed somites. It is one of the first reported *Dmrt* genes expressed outside the gonads (Meng et al., 1999). In this organism overexpression of Terra induces apoptosis in the somitic mesoderm, suggesting that *Terra* expression levels need to be tightly regulated for proper mesoderm development.

During chick embryogenesis, in addition to the developing somites, *Terra* is transiently expressed on the left side of the Hensen's node, a pattern highly suggestive of a role in left–right axis determination. Genes that show a similar asymmetric distribution at this stage include *Shh*, *Nodal* and *HNF3β*, and these factors have been shown to play an important role in the establishment of left–right asymmetry (reviewed in Raya and Izpisúa Belmonte, 2006). This possibility was recently tested in the zebrafish embryo using a morpholino-mediated knockdown approach (Saude et al., 2005). During formation of the vertebrate body plan, it is fundamental to create left–right asymmetry in the lateral mesoderm to correctly position the organs on each side of the midline. Terra-morpholino-injected zebrafish embryos displayed a randomization of left sided-specific genes, which correlated with a randomization of the heart position, consistent with a role for *Terra* in left–right axis determination (Saude et al., 2005). Moreover, these embryos exhibited a desynchronization of the segmentation clock in the mesoderm, which is essential for normal development of bilateral structures such as skeletal muscles. This desynchronization resulted in the formation of extra somites in zebrafish morphants (Saude et al., 2005). As such, *Terra* is a key factor linking left–right asymmetry to the bilateral synchronization of the segmentation clock in the mesoderm. However, *Terra* has not been shown to be asymmetrically distributed in the zebrafish embryo, and it remains to be determined whether *Terra* is also functioning in the same pathway in birds. Finally, a bilateral defect has not been reported in mouse embryos lacking *Dmrt2* function (Seo et al., 2006), which suggests that this activity of *Terra/Dmrt2* may not have been conserved during evolution.

Mouse embryos lacking *Dmrt2* die early, which makes it difficult to determine whether *Dmrt2* has a function in post-natal gonadal development (Seo et al., 2006). However, both male and female homozygous embryos were obtained at equal frequency suggesting that *Dmrt2* is not required for sex determination. This further supports the view that deletion of a single DMRT gene present in the distal region of human chromosome 9 is not sufficient to elicit sex reversal (Ottolenghi et al., 2000), and that the combined loss of activity of multiple

Table 1
Developmental expression of members of the *Dmrt* gene family across species

Genes	Species	Gonads	Embryonic expression in non-gonadal tissues	Methods	References
Dmrt1	Human	T	nr	ISH	Moniot et al., 2000
	Mouse	IG→T	nd	ISH	Raymond et al., 1999a; Smith et al., 1999
	Wallaby	O, T	nr	IHC	Pask et al., 2003
	Platypus	T	nr	PCR	El-Mogharbel et al., 2007
	Chicken	IG→T	nr	ISH	Raymond et al., 1999a; Shan et al., 2000; Smith et al., 1999
	Alligator	O, T	nr	PCR	Smith et al., 1999
	Lizard	IG→T	nr	PCR, ISH	Sreenivasulu et al., 2002
	Turtle	O, T	nr	PCR, ISH	Kettlewell et al., 2000
	Xenopus	O, T	nr	PCR, ISH	Osawa et al., 2005
	Rana	T	nr	PCR, IHC	Shibata et al., 2002; Aoyama et al., 2003
(*Dmy*)	Medaka	T	nd	PCR, ISH	Matsuda et al., 2002; Nanda et al., 2002; Kobayashi et al., 2004
	Platyfish	T	nd	PCR, ISH	Veith et al., 2003; Veith et al., 2006a
	Zebrafish	O, T	nr	PCR, ISH	Guo et al., 2005
	Rainbow trout	T	nr	N	Marchand et al., 2000
	Rice field eel	O, T	nr	PCR, ISH	Huang et al., 2005a
Dmrt2	Human	T	nr	PCR	Ottolenghi et al., 2000
	Mouse	T	somite	PCR, ISH	Meng et al., 1999; Ottolenghi et al., 2000; Kim et al., 2003; Seo et al., 2006
	Platypus	O, T	nr	PCR	El-Mogharbel et al., 2007
	Chicken	nr	Hensen's node, somite, eye, otic vesicle	ISH	Saude et al., 2005
	Rana	IG→T	nr	PCR	Matsushita et al., 2007
	Medaka	IG→T	somite, head	ISH	Winkler et al., 2004
	Platyfish	nd	somite, branchial arches	ISH	Veith et al., 2006a
(*Terra*)	Zebrafish	nr	somite	ISH	Meng et al., 1999
Dmrt3	Human	T	nr	PCR	Ottolenghi et al., 2002
	Mouse	IG→T	nasal placode, telencephalon, spinal cord	ISH, PCR	Smith et al., 2002; Kim et al., 2003
	Platypus	O, T	nr	PCR	El-Mogharbel et al., 2007
	Chicken	nr	nasal placode, telencephalon, spinal cord, somite, branchial arches, mullerian duct	ISH	Smith et al., 2002
	Rana	IG→T	nr	PCR	Matsushita et al., 2007
	Medaka	IG→T	spinal cord	PCR, ISH	Winkler et al., 2004
Dmrt4	Human	nd	nr	PCR	Ottolenghi et al., 2002
	Mouse	O, T	ubiquitous	PCR, ISH	Kim et al., 2003; Balciuniene et al., 2006
	Xenopus	T	nasal placode, telencephalon, foregut, gall bladder	ISH	Huang et al., 2005b
	Medaka	IG→T	nasal placode, telencephalon, otic placode, branchial arches	PCR, ISH	Kondo et al., 2002; Winkler et al., 2004
	Platyfish	nd	nasal placode, forebrain, branchial arches	ISH	Veith et al., 2006a
Dmrt5	Human	T	nr	PCR	Ottolenghi et al., 2002
	Mouse	O, T	brain	PCR, ISH	Kim et al., 2003
	Rana	O, T	nr	PCR	Matsushita et al., 2007
	Platyfish	nd	nasal placode, lens, forebrain, midbrain	ISH	Veith et al., 2006a
	Zebrafish	O, T	midbrain, midbrain–hindbrain boundary	ISH	Guo et al., 2004

Table 1 (*continued*)

Genes	Species	Gonads	Embryonic expression in non-gonadal tissues	Methods	References
Dmrt6	Human	O, T	nr	PCR	Ottolenghi et al., 2002
	Mouse	O, T	brain	PCR	Kim et al., 2003
Dmrt7	Human	T	nr	PCR	Ottolenghi et al., 2002
	Mouse	O, T	nd	PCR, ISH, IHC	Kim et al., 2003; Kawamata and Nishimori, 2006; Kim et al., 2007a
Dmrt8	Human	O, T	nr	PCR	Ottolenghi et al., 2000
	Mouse	IG→T	nr	PCR, ISH	Veith et al., 2006b

O, ovary; T, testis; IG→T, indifferent gonad and maintained in testis; nr, not reported; nd, not detected; ISH, in situ hybridization; N, Northern; PCR, polymerase chain reaction; IHC, Immunohistochemistry.

DM family members might be a prerequisite for expression of this phenotype (reviewed in Ottolenghi and McElreavey, 2000).

In the mouse embryo *Dmrt2* is expressed in the presomitic mesoderm (Meng et al., 1999) and in the dermomyotome of the somites (Seo et al., 2006). Mouse embryos lacking *Dmrt2* function die soon after birth of respiratory distress. After closer examination, these mutant embryos showed abnormal rib and sternal development. The loss of *Dmrt2* leads to embryonic somite patterning defects, first visible at E10.5 and more pronounced by E11.5. Both the dermomyotome and myotome fail to adopt a normal epithelial morphology in the absence of *Dmrt2*. Associated with these morphological defects, alterations in the expression of dermomyotomal and myotomal transcription factors such as *Pax3*, *Paraxis*, *Myf5*, *Myogenin*, *Mrf4* and *MyoD* were observed. The skeletal phenotype appears to occur independently of any changes in cell proliferation or cell death in these progenitor cells (Seo et al., 2006). This is in contrast to studies in the zebrafish, where changes in *Terra* expression levels have been linked to apoptosis in the mesoderm (Meng et al., 1999). Interestingly, despite these early defects, mouse embryos harvested from E13.5 onwards exhibited relatively normal muscle pattern and mass, suggesting that early defects are compensated later in embryogenesis, presumably by the activity of another *Dmrt* gene expressed in the somites or alternatively by a mechanism independent of *Dmrt* function (Seo et al., 2006).

Studies in fish and mouse embryos indicate that *Terra/Dmrt2* has an essential function in muscle development. However, the type of developmental processes regulated by this *Dmrt* family member appear to be distinct in these organisms.

Dmrt4 has distinct activities in frog and mouse

In Xenopus *Dmrt4* is detected early in the presumptive olfactory placode, but appears to be dispensable for olfactory placode induction. Embryos injected with a Dmrt4-specific morpholino antisense oligonucleotide can still activate a broad range of nasal placode marker genes (Huang et al., 2005b). The compensatory activity of other *Dmrt* family members, such as *Dmrt3* or *Dmrt5* could account for this lack of defect in olfactory placode specification.

Despite the lack of an early phenotype, Xenopus Dmrt4-depleted embryos showed a strong and specific loss of Xebf2, an HLH transcription factor implicated in neuronal differentiation. Later in embryogenesis, morpholino-injected embryos exhibited reduced neurogenesis in the olfactory epithelium (Huang et al., 2005b). In gain of function experiments, Xenopus *Dmrt4* has been shown to promote the expression of neural genes in naïve ectoderm through the activation of *Neurogenin* and *Xbef2* (Huang et al., 2005b), suggesting a central role for *Dmrt4* in regulating neurogenesis. An important follow up to these experiments would be to determine whether *Neurogenin* and *Xebf2* are direct targets of *Dmrt4*, and whether this function has been conserved in other species.

Dmrt4 null mice develop essentially normally, undergo full sexual differentiation in both sexes and are completely fertile, indicating that *Dmrt4* is not required for the development of the mouse gonads (Balciuniene et al., 2006). However, two phenotypes have been described in *Dmrt4* mouse mutants. First, the ovaries of most mutant females present polyovular follicles, suggesting that *Dmrt4* regulates folliculogenesis, a process during which oocytes are incorporated into primordial follicles. Second, mutant males exhibited mounting behavior toward other males. While observed at a rather low frequency, this behavior was consistently observed in multiple rounds of testing (Balciuniene et al., 2006). Olfaction and sexual behavior are strongly linked in mammals (Keverne, 2004), nevertheless there was no significant alteration in the nasal placodes of *Dmrt4* deficient animals. Moreover, histologically and functionally (based on food and pheromones detection assays) the adult olfactory epithelium of mutant animals appeared to be normal (Balciuniene et al., 2006).

The expression of *Dmrt4* differs greatly between frog and mouse (Huang et al., 2005b; Balciuniene et al., 2006). In fact, based on expression pattern Xenopus *Dmrt4* is more closely related to mouse *Dmrt3* (Smith et al., 2002). This could account for some of the differences observed in the phenotypes of mouse and Xenopus lacking *Dmrt4* function; Xenopus in which *Dmrt4* function appears to be critical for neurogenesis in the olfactory system (Huang et al., 2005b). Mouse *Dmrt4* is expressed (but not restricted) in the developing nasal placode (Balciuniene et al., 2006) and in

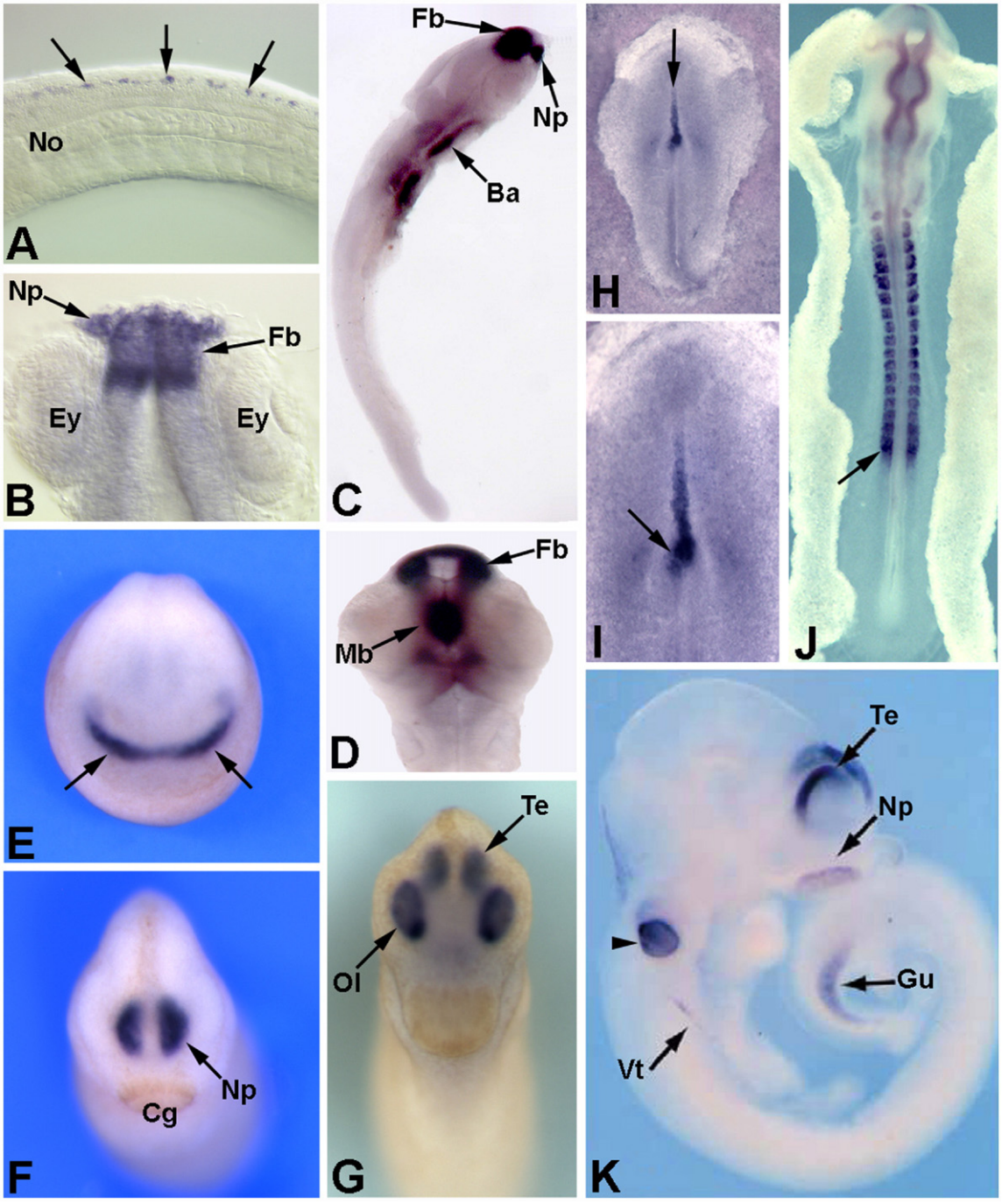

Fig. 1. Whole-mount in situ hybridization analysis of *Dmrt* genes expression in fish, frog, chick and mouse embryos. (A) Clusters of *Dmrt3* positive cells (possibly dorsal interneurons) are detected in the spinal cord of a stage 31 (4 dpf) medaka embryo (arrows). Lateral view, dorsal to top. (B) *Dmrt4* expression in the forebrain (Fb) and nasal placode (Np) of a stage 22 (1.5 dpf) medaka embryo. Dorsal view, anterior to top. (C) In a stage 11 (3.9 dpf) platyfish embryo Dmrt4 is detected in the forebrain (Fb), nasal placodes (Np) and branchial arches (Ba). Lateral view, anterior to top. (D) Dmrt5 is expressed in the forebrain (Fb) and in the midbrain (Mb) of a stage 12 (4.2 dpf) platyfish embryo. Dorsal view, anterior to top. (E) *Dmrt4* expression in the prospective nasal placode (arrows) of a stage 15 Xenopus embryo. (F) After neural tube closure (stage 25) Xenopus *Dmrt4* expression domain is restricted to the two nasal placodes (Np). (G) At stage 35 Xenopus *Dmrt4* is detected in the telencephalon (Te) and in the olfactory organs (Ol). For panels (E–G), anterior views, dorsal to top. (H, I) In a stage 5+HH chick embryo *Terra/Dmrt2* is expressed in Hensen's node and in the elongating notochord (arrow). Dorsal view, anterior to top. (I) Higher power of the embryo shown in (H) illustrating the asymmetric expression of *Terra* on the left side of Hensen's node (arrow). (J) Terra expression in the presomitic mesoderm (arrow) and somites of a 15-somite stage chick embryo. Dorsal view, anterior to top. (K) In E10.5 mouse embryo *Dmrt3* is expressed in the nasal placode (Np), telencephalon (Te), post-anal gut (Gu) and vagal tract outflow (Vt). Staining in the otic vesicle (arrow head) is non-specific. Lateral view, anterior to top. Cg, cement gland; Ey, eye; No, notochord. Photographs of medaka, platyfish, chick and mouse in situ hybridization courtesy of Drs. C. Winkler, A-M. Veith, L. Saude and C. Smith, respectively. All images are original in situ hybridization pictures, with the exception of panel (K), modified from Smith et al. (2002).

Table 2
Major phenotypes associated with loss or gain of function of *Dmrt* genes

Genes	Species	LOF/GOF	Phenotypes	References
Dmrt1	Human	M	Gonadal dysgenesis and XY sex reversal[(a)]	Veitia et al., 1997
			Gonadal dysgenesis and XY sex reversal[(a)]	Raymond et al., 1999b
			Variable XY sex reversal and mental retardation[(a)]	Muroya et al., 2000
			Ovotestes in genetic males and growth retardation[(a)]	Ounap et al., 2004
		KO	Failure to differentiate (embryonic carcinoma cell line)	Koji et al., 2006
	Mouse	KO	Pre-meiotic germ cells fail to undergo radial migration and to survive. Failure of Sertoli cells differentiation	Raymond et al., 2000; Kim et al., 2007b
(*Dmy*)	Medaka	M	Female development in genetic males	Matsuda et al., 2002
		GOF	Male development in genetic females	Matsuda et al., 2007
Dmrt2	Human	M	Gonadal dysgenesis and XY sex reversal[(a)]	Raymond et al., 1999b
			Variable XY sex reversal and mental retardation[(a)]	Muroya et al., 2000
			Ovotestes in genetic males and growth retardation[(a)]	Ounap et al., 2004
	Mouse	KO	Embryonic somite patterning defects	Seo et al., 2006.
(*Terra*)	Zebrafish	GOF	Increased apoptosis	Meng et al., 1999
		KD	Randomization of left-sided-specific genes and desynchronization of the segmentation clock	Saude et al., 2005
Dmrt4	Mouse	KO	Females with polyovular follicles and males with copulatory behavior toward other males	Balciuniene et al., 2006
	Xenopus	KD	Impaired neurogenesis in the olfactory epithelium	Huang et al., 2005b
		GOF	Promote neurogenesis in naïve ectoderm	Huang et al., 2005b
Dmrt7	Mouse	KO	Infertility with spermatogenic arrest at pachytene stage and abnormal sex chromatin modifications	Kawamata and Nishimori, 2006; Kim et al., 2007a

GOF, Gain of function; KD, Knockdown; KO, Knockout; LOF, loss of function; M, Mutation. (a), patients with deletion of a region of chromosome 9p containing DMRT1, DMRT2 and DMRT3 genes.

this tissue it overlaps with *Dmrt3* (Smith et al., 2002), it is therefore possible that *Dmrt3* compensate for the loss of *Dmrt4* in these mutants, suggesting that the functions of these genes may have shifted along with their expression patterns during vertebrate evolution.

The broad expression of *Dmrt4* in the mouse (Balciuniene et al., 2006) makes genetic redundancy a possibility in a number of tissues. However, *Dmrt4* is not functioning redundantly with other gonadally expressed DM domain genes, *Dmrt1* and *Dmrt7*, since no enhancement of the gonadal phenotypes was observed in the double (*Dmrt1/Dmrt4* or *Dmrt7/Dmrt4*) mutants (Balciuniene et al., 2006). This indicates that at least in the gonad these genes function independently.

Dmrt7 is essential for male fertility

While most members of the *Dmrt* gene family are expressed at higher level in the developing testis than in the ovary, *Dmrt7* is more abundant in the embryonic females gonads than in the testis (Kim et al., 2003). This expression in the ovary is independent of the germ line, since in XX c-kit mutants, which lack germ cells, the levels of expression of Dmrt7 was largely unchanged. However in c-kit mutant testes Dmrt7 expression appears to be germline dependent (Kim et al., 2003). Postnatally *Dmrt7* becomes male-specific (Kawamata and Nishimori, 2006; Kim et al., 2007a).

Recently, *Dmrt7*-deficient mouse have been generated by gene targeting (Kawamata and Nishimori, 2006; Kim et al., 2007a). These animals are born and are indistinguishable from their wild-type littermates. They lived into adulthood suggesting that *Dmrt7* is not required for embryonic development. While females showed normal fertility, adult null males were infertile. No sperm was detected in the fluid prepared from the epididymis of *Dmrt7* mutant males. The cause of infertility in these animals is believed to be due to an arrest of spermatogenesis at the late pachytene stage (Kawamata and Nishimori, 2006; Kim et al., 2007a). *Dmrt7* function is exclusively required in the germ line, since animals with conditional inactivation of *Dmrt7* in Sertoli cells have normal testis and spermatogenesis (Kim et al., 2007a). This suggests that aberrant Sertoli cells organization observed in Dmrt7 deficient animals is an indirect consequence of the lack of *Dmrt7* in germ cells. Loss of *Dmrt7* is also associated with abnormal sex chromatin modifications, normally required for male meiotic progression (Kim et al., 2007a).

Conclusions and perspectives

There is strong evidence that homologues of the *doublesex* gene of Drosophila and the *mab-3* gene of *C. elegans* have retained a conserved function in sexual development during evolution. This appears to be true throughout the animal kingdom. For example, a DM domain-containing gene has been identified in the cnidarian Acropora millepora (*AmDM1*), and interestingly, in this organism *AmDM1* transcripts accumulate during sex cell differentiation, also consistent with a role in sexual development (Miller et al., 2003).

Since the identification of the first mammalian DM-domain containing gene, *Dmrt1* (Raymond et al., 1999a), it has been unclear whether this gene family is exclusively involved in gonadal development. Recent studies indicate that *Dmrt* genes belong to a family of important developmental regulators,

providing evidence that this gene family has evolved to adopt functions in developmental pathways distinct from that of sex determination or differentiation (Meng et al., 1999; Huang et al., 2005b; Saude et al., 2005; Balciuniene et al., 2006; Seo et al., 2006). Obviously, a great deal still needs to be learned about the precise function of *Dmrt* genes in and outside the developing gonads. Several of the *Dmrt* genes expressed in the developing gonad appear to share similar functional characteristics. However, in non-gonadal tissues *Dmrt* factors appear to have the ability to regulate a broad range of developmental processes.

Although the Drosophila DM domain proteins dsx^M and dsx^F act as a transcription activator and repressor, respectively (Saccone et al., 2002), very little is known about the transcriptional control mediated by vertebrate *Dmrt* genes. Moreover, the downstream targets of *Dmrt* genes in vertebrates remain quite elusive. The identification of such targets represents one of the important challenges for the years to come to further our understanding of this important class of molecules during embryogenesis.

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