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REVIEW

Expanding roles for the evolutionarily conserved *Dmrt* sex transcriptional regulators during embryogenesis

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Abstract *Dmrt* genes encode a large family of transcription factors characterized by the presence of a DM domain, an unusual zinc finger DNA binding domain. While *Dmrt* genes are well known for their important role in sexual development in arthropodes, nematodes and vertebrates, several new findings indicate emerging functions of this gene family in other developmental processes. Here, we provide an overview of the evolution, structure and mechanisms of action of *Dmrt* genes. We summarize recent findings on their function in sexual regulation and discuss more extensively the role played by these proteins in somitogenesis and neural development.

Keywords *Dmrt* · Sexual differentiation · Somitogenesis · Neurogenesis · Olfactory placode · Telencephalon

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Introduction

The *Dmrt* (*d*oublesex and *m*ab3-*r*elated-*t*ranscription factor) family derives its name from two founding members, *doublesex* (*dsx*) in *Drosophila melanogaster* and *male abnormal* (*mab-3*) in *Caenorhabditis elegans*. It is defined by the presence of the DM domain DNA binding motif, an unusual cysteine-rich zinc DNA binding motif constituted of two intertwined zinc fingers [1, 2]. *Dmrt* genes are well known to play a conserved role in sex determination, sexual dimorphism or other aspects of sexual reproduction [3, 4], but a growing body of evidence indicates that they are also conserved regulators of other developmental processes [5]. In this review, we provide an updated overview of the evolution, structure and mechanisms of action of DM genes. We summarize recent findings on their function in sexual development and discuss more extensively recent functional studies demonstrating their important function in somitogenesis and neural development. In particular, we highlight the important role of a subgroup of them including *Dmrt3*, *Dmrt4* and *Dmrt5*, characterized by the presence of an additional highly conserved domain designated DMA, in neurogenesis and patterning of the developing nervous system.

Taxonomic distribution and architecture of Dmrt proteins

Although DM domain genes have been extensively studied in model organisms, little is known about the evolution of this gene family. DM domain genes have been detected in many bilaterian species, including in the amphioxus [6], the oyster *Crassostrea gigas* [7], and in two cnidarian species, the coral *Acropora millepora* [8] and the sea anemone *Nematostella vectensis* [9]. Nevertheless, the evolutionary origin of *Dmrt* genes in animals has not been further investigated due to a

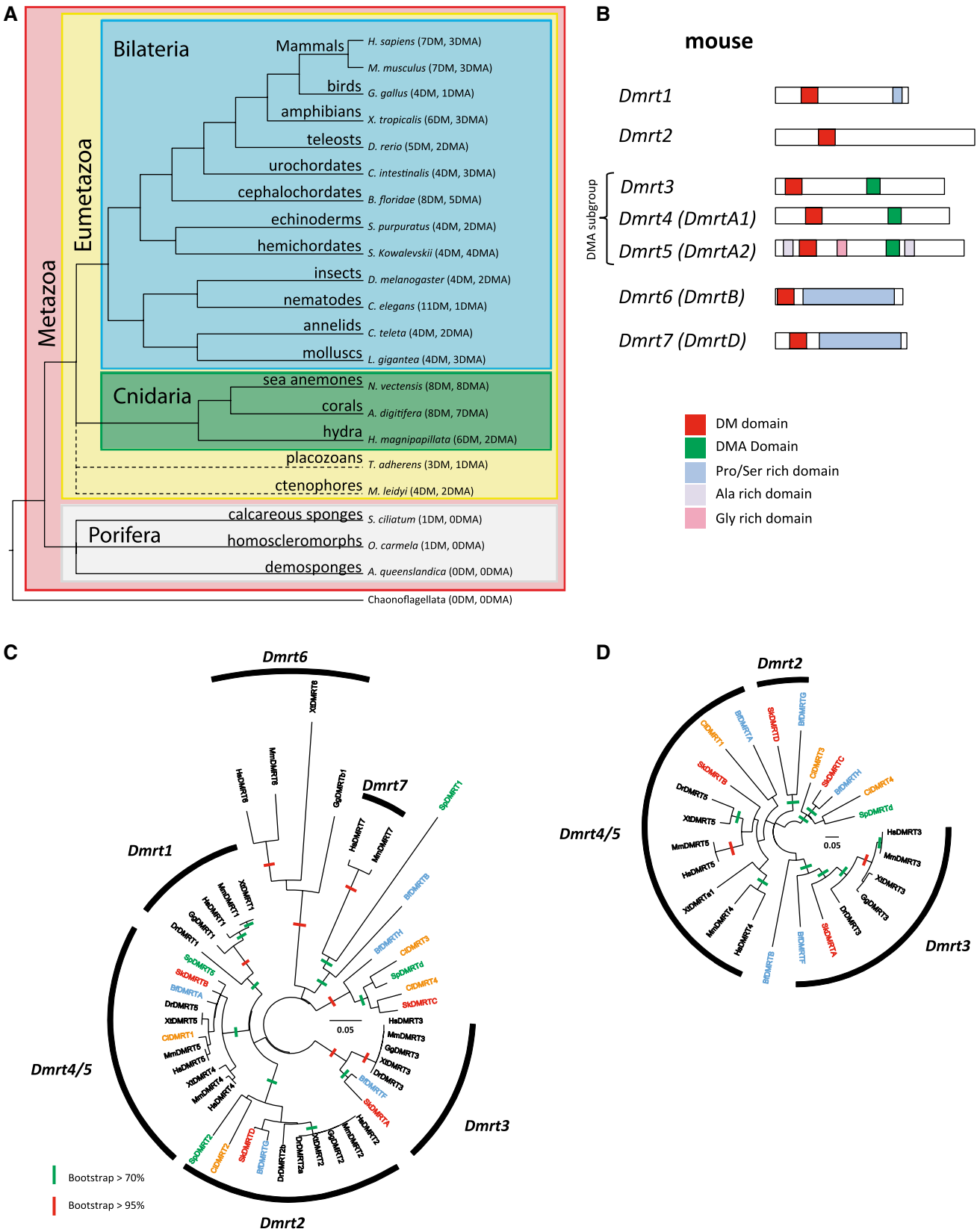
lack of data from basal metazoans. Using available whole-genome sequences from several basal metazoans, we identified novel DM genes in the cnidarian *Acropora digitifera*, in the placozoan *Trichoplax adhaerens*, in the ctenophore *Mnemiopsis leidyi* and in the sponges *Sycon ciliatum* and *Oscarella carmella* (Fig. 1a; Suppl. Table 1). However, DM domain genes were not detected in the genome of the sponge *Amphimedon queenslandica*, and in several non-metazoan holozoans including choanoflagellates, the closest relatives to metazoans. Thus, the DM domain gene family probably arose during early metazoan evolution after the divergence with choanoflagellates, and expanded in the metazoan lineage.

Vertebrates have multiple *Dmrt* genes (e.g., *Dmrt1* to *Dmrt8* in mice and humans). Dmrt proteins have been classified into several subfamilies, based on the presence of additional conserved protein domains and on the exon–intron structure of the corresponding genes. *Dmrt4* (*Dmrta1*), *Dmrt5* (*Dmrta2*) and *Dmrt3* (*Dmrta3*) constitute one such subfamily characterized by the presence of a conserved DMA domain at their C-termini (Fig. 1b). Each gene of this subfamily has two coding exons, with the DM domain encoded by the first coding exon and the DMA domain encoded by the second one [10]. In *N. vectensis*, a DMA domain is present in all eight *Dmrt* genes, suggesting that its presence may represent the ancestral condition for cnidarians and bilaterians [9]. We observed the presence of a DMA domain in some or all the *Dmrt* genes in most metazoan species we looked at. This includes non-bilaterians such as the placozoan *Trichoplax* and the ctenophore *Mnemiopsis* (Fig. 1a; Suppl. Table 1), indicating that *Dmrt* genes with both DM and DMA domains were already present during early metazoan evolution. Uncertainties about the phylogenetic relationships of non-bilaterian animals [11, 12] and the relative paucity of genomic data from non-bilaterian species do not allow the drawing of firm conclusions about whether a DM + DMA architecture may represent the ancestral state of *Dmrt* genes in metazoans. Further studies are also required to define whether the last common ancestor of bilaterians and that of bilaterians and cnidarians only owned *Dmrt* genes with DM and DMA domains, as is observed in some present-day species (such as *Nematostella* and *Saccoglossus*), or whether both genes with and without DMA domain coexisted in these remote ancestors, as found in many extant species (such as vertebrates).

Phylogeny of the *Dmrt* genes

We performed a phylogenetic analysis of the Dmrt sequences to study the evolution of individual *Dmrt* gene members. The phylogenetic relationship among Dmrt proteins is not obvious, as there is little sequence similarity outside the short DM and DMA domains. Here, we limited our study to deuterostomes including sequences from various chordates, echinoderms and hemichordates. A thorough phylogenetic analysis is needed to draw conclusions about the origin and the evolution of the *Dmrt* family at the level of the metazoans. Phylogenetic analyses of both DM and DMA domains (Fig. 1c, d) suggest that the *Dmrt2*, *Dmrt3* and *Dmrt4/5* vertebrate subgroup is monophyletic and that orthologs of each of these genes were already present in the ancestor of deuterostomes and contained both DM and DMA domains. Comparison between DM and DMA domain phylogenies suggest that vertebrate *Dmrt2* have lost their DMA domain since related sequences in cephalochordates and hemichordates contain this domain. Vertebrate *Dmrt1*, *Dmrt6* and *Dmrt7* also appear to be monophyletic. They could not be related unambiguously to non-vertebrate deuterostome sequences or other *Dmrt* vertebrate subgroups due to weak phylogenetic signal. The question of their origin remains unsolved. *Dmrt8* (*Dmrtc1*) show strong similarity over their entire sequence to *Dmrt7* (*Dmrtc2*) but lack the DM domain [13]. Both have been found in mammals but not in other vertebrate species. In *Dmrt8* from human, chimp and orangutan, the open reading frame is disrupted by a stop codon 5′ of the DM

Fig. 1 Taxonomic distribution and phylogeny of the *Dmrt* genes and domain architecture of the proteins. **a** Distribution of *Dmrt* genes in representatives of metazoans. For each species, the number of Dmrt paralogs, with or without a DMA domain, is reported in parentheses. Accession numbers of the sequences included in this analysis are provided in Suppl. Table S1. Metazoa are highlighted in *red*, Eumetazoa in *yellow*, Bilateria in *blue*, Cnidaria in *green* and Porifera in *gray*. The phylogenetic relationships of placozoans and ctenophores with the other metazoan groups are still controversial and are therefore represented by *dashed lines*. In some phylogenetic studies [12], ctenophores together with cnidarians constitute the sister group to bilaterians, while in other studies [11], ctenophores are found as the sister group to all other metazoans including sponges. Similarly, placozoans have been reported either as belonging to eumetozoans [12], or as the sister group to all other metazoans [102]. **b** Domain architecture of the mouse Dmrt paralogs. For each Dmrt protein, the additional names are provided. Dmrt proteins share highly conserved protein motifs, including the DM domain, and are subdivided into a few subgroups based on sequence similarity. *Dmrt3*, *Dmrt4*, and *Dmrt5* are categorized as a subgroup because their products share a highly conserved DMA domain at their C-termini. Pro/Ser-, Ala- and Gly-rich domains are also indicated. **c**, **d** Phylogenetic relationships between deuterostomian Dmrts inferred by neighbor-joining (NJ) analyses of the DM (**c**) and DMA (**d**) domains. Sequences included belong to the major groups of deuterostomes: hemichordates (sequence names in *red*), echinoderms (*green*), cephalochordates (*blue*), urochordates (*orange*) and vertebrates (*black*); *Bf*, *Branchiostoma floridae*; *Dr*, *Danio rerio*; *Hs*, *Homo sapiens*; *Mm*, *Mus musculus*; *Gg*, *Gallus gallus*; *Sk*, *Saccoglossus kowalevskii*; *Sp*, *Strongylocentrotus purpuratus*; *Ci*, *Ciona intestinalis*; *Xt*, *Xenopus tropicalis*. See Table S1 for information about sequences. For each genomes, all identified sequences have been included except the very divergent *B. floridae* DmrtC, DmrtD and DmrtE. Branches crossed by a *green* or *red bar* indicate bootstrap value ≥70 and ≥95 %, respectively (1,000 NJ bootstrap replicates)


A
Metazoa
Eumetazoa
Bilateria
Mammals
H. sapiens (7DM, 3DMA)
M. musculus (7DM, 3DMA)
birds
G. gallus (4DM, 1DMA)
amphibians
X. tropicalis (6DM, 3DMA)
teleosts
D. rerio (5DM, 2DMA)
urochordates
C. intestinalis (4DM, 3DMA)
cephalochordates
B. floridae (8DM, 5DMA)
echinoderms
S. purpuratus (4DM, 2DMA)
hemichordates
S. Kowalevskii (4DM, 4DMA)
insects
D. melanogaster (4DM, 2DMA)
nematodes
C. elegans (11DM, 1DMA)
annelids
C. teleta (4DM, 2DMA)
molluscs
L. gigantea (4DM, 3DMA)
Cnidaria
sea anemones
N. vectensis (8DM, 8DMA)
corals
A. digitifera (8DM, 7DMA)
hydra
H. magnipapillata (6DM, 2DMA)
placozoans
T. adherens (3DM, 1DMA)
ctenophores
M. leidyi (4DM, 2DMA)
Porifera
calcareous sponges
S. ciliatum (1DM, 0DMA)
homoscleromorphs
O. carmela (1DM, 0DMA)
demosponges
A. queenslandica (0DM, 0DMA)
Chaonoflagellata (0DM, 0DMA)
B
mouse
Dmrt1
Dmrt2
DMA subgroup
Dmrt3
Dmrt4 (DmrtA1)
Dmrt5 (DmrtA2)
Dmrt6 (DmrtB)
Dmrt7 (DmrtD)
DM domain
DMA Domain
Pro/Ser rich domain
Ala rich domain
Gly rich domain
C
Dmrt6
Dmrt7
Dmrt1
Dmrt4/5
Dmrt3
Dmrt2
0.05
Bootstrap > 70%
Bootstrap > 95%
D
Dmrt2
Dmrt4/5
Dmrt3
0.05

domain. Therefore, primate *Dmrt8* genes do not have the potential to encode a DM domain [14].

Mechanisms of action of Dmrt proteins

Despite the importance of Dmrt proteins, how they regulate the expression of specific target genes remains elusive. *Dmrt3* and *Dmrt5* have been shown to encode nuclear proteins [9, 15]. Sequence-specific DNA binding via the DM domain has been determined in vitro for most vertebrate Dmrt proteins [9, 15, 16]. They all bind very similar DNA sequences resembling those bound by DSX [17, 18] and MAB-3 [19]. The consensus consists in a palindromic sequence (G/A)NNAC(A/T)A(A/T)GTNN(C/T) composed of two half-sites around a central (A/T) base pair. As predicted from the symmetric nature of this site, Dmrt proteins bind DNA as homodimers or heterodimers with other DM domain proteins [16, 17].

DM domain proteins are able to act as transcriptional activators or repressors. Reporter gene transcription assays and deletion analysis have indicated that their regulatory domains are located at their C-termini [9, 20, 21]. To date, transcriptional regulation by Dmrt proteins has only been investigated at a large scale in the case of Dmrt1 in mouse sexual differentiation (see below). In cell cultures, Dmrt1 activates or represses transcription depending on cell type and promoter structure. In vivo, in the developing mouse male and female gonads, Dmrt1 acts simultaneously at multiple sites across the genome, activating some genes and repressing others. Thus, Dmrt1 functions as a bifunctional transcriptional regulator. It also binds its own promoter as well as one of the other *Dmrt* genes, suggesting auto- and cross-regulation of these genes. Chromatin immunoprecipitation (ChIP) analysis using conditional mutant testes showed that DNA binding and transcriptional regulation of individual target genes can differ between germ cells and Sertoli cells, and that some genes exhibit Dmrt1 binding only in one cell type. Differential response of genes to loss of Dmrt1 appears to correspond to differences in the binding motif, suggesting that other transactivating factors modulate its activity [22–25]. Whether other DM proteins function like Dmrt1 as bifunctional transcriptional regulators is unclear. MAB-3 represses transcription of yolk gene (*vitellogenin*) in the intestine and blocks transcription of the antineural bHLH gene *ref-1*, a repressor of the proneural protein lin-32 in male ray precursor cells [19, 26]. *dsx* is alternatively spliced into sex-specific isoforms that encode proteins that share the DM domain but have distinct C-termini. DSX^M in males blocks, whereas DSX^F in females activates, transcription of genes such as the female-specific yolk protein genes or the *bric a brac 1* (*bab1*) and *bab2* genes which control pigmentation [27–29]. Recently, novel DSX targets during genital development have been identified, some being activated and others being repressed by DSX [30]. However, it is not known whether these novel downstream genes are direct or indirect targets. Thus, so far, only Dmrt1 has been shown to be bifunctional.

How do Dmrt proteins achieve transcription repression or activation in a cell-specific manner? In contrast to other zinc finger proteins, DM proteins interact with DNA in the minor groove rather than the major groove [2, 31]. This enables them to bind to DNA on sites overlapping those of major groove binding proteins and to physically interfere with their binding or to cooperate with them. Such a mechanism has been observed in the case of DSX and MAB-3 [26, 28, 29, 32, 33]. Whether this mechanism is also used by Dmrt proteins in vertebrate is not known. Whether they recruit coactivators or corepressors and associate with chromatin-modifying enzymes also remains to be investigated.

Dmrts are conserved regulators of sexual development

DM domain-containing genes are conserved genetic components involved in sex differentiation in all animals that have been studied. In flies, *dsx* act downstream in the sex-determination pathway. *dsx* is expressed in somatic gonadal primordium and its function in the somatic gonad is required for sex-specific germline development. Later in development, *dsx* is widely expressed in a subset of non-gonadal cells where it acts cell autonomously and non-cell autonomously, via interactions with conserved *Hox* genes and signaling pathways, to integrate sex-specific, spatial and temporal cues and induce localized sex-specific differentiation. Three other DM domain genes are present in flies, but only *dsx* has been shown to regulate sexual dimorphism.

Caernorhabditis elegans has 11 DM domain genes. Among them, 3 are known to regulate sexual development: *mab-3*, *mab-23* and *DM domain 3* (*dmd-3*). They are dispensable to gonad development but are involved in the sexual differentiation of somatic tissues including copulatory structures such as sensory rays, spicules and mating muscles. As in flies, DM domain genes in nematodes integrate sexual and various positional and temporal cues to initiate a sex-specific developmental program (reviewed in [3, 4]).

In *Daphnia magna*, a freshwater branchiopod crustacean which parthenogenetically produces males in response to environmental cues, a DM domain gene, *DapmaDsx1*, has been identified. *DapmaDsx1* shows higher expression in male-specific structures. Knockdown of *DapmaDsx1* in male embryos directs the production of female traits whereas ectopic expression of *DapmaDsx1* in female results in the development of male-like phenotypes [34].

In *Acropora millepora*, transcripts of the DM domain gene *AmD1* are present at higher levels during sexual differentiation [8], suggesting that the implication of *Dmrt*

genes in sexual regulation may have predated the divergence between cnidarians and bilaterians.

In vertebrates, all *Dmrt* family members are expressed in the indifferent gonad and in most cases they are subsequently maintained at higher levels in male as opposed to female gonads (Table 1). Among the eight DM domain genes in vertebrates, three of them have been established to have roles in gonad and/or germ line development, namely *Dmrt1*, *Dmrt4* and *Dmrt7* (Table 2). In the following section, we briefly highlight the functions of two of them, *Dmrt1* and *Dmrt7*, and show that *Dmrt1* or a close paralog has recently moved up the regulatory hierarchy from downstream positions in gonad and germ line development to the top level of sex determination during evolution in fish, birds and frogs. The role of *Dmrt4* in gonadal and non-gonadal tissues is discussed in the next section. Recent excellent reviews have more extensively discussed the role of Dmrts in the development and evolution of sex dimorphism [3, 4, 35, 36].

Dmrt1 triggers male-specific and represses female-specific differentiation

In humans, *Dmrt1* is localized in a region on the short arm of chromosome 9 (9p24.3) including three *Dmrt* genes (*Dmrt1–3*). Deletions of this region are associated with testicular dysgenesis and, in some cases, cause sex reversal of the XY embryonic gonad into ovarian tissue [37–40]. Among the three genes, *Dmrt1* is the strongest candidate for XY gonadal dysgenesis. It is indeed expressed in the human embryonic genital ridges of human male, but not female embryos [41]. Deletions or mutations of *Dmrt1* can cause XY gonadal dysgenesis [42–45]. Therefore, *Dmrt1* was thought to have an important function in sex determination. Feminization associated with the loss of *Dmrt1* is, however, more likely due to a failure of male gonadal differentiation or to the reprogramming of Sertoli cells into granulosa-like cells, as recently discovered in mice (see below). *Dmrt1* is also associated with testicular germ cell cancer [46–48], which is consistent with a role of *Dmrt1* as a tumor suppressor in 129v mice [22].

In mouse embryos, *Dmrt1* is expressed in the genital ridge of both sexes before any overt signs of sex differentiation. Later, *Dmrt1* expression declines in the ovary but is maintained in the testis, where it is restricted to premeiotic germ cells and Sertoli cells [49]. In mice, Dmrt1 is not required for primary sex determination as XY *Dmrt1* mutants are born as males. It is, however, involved in multiple aspects of the male gonad differentiation. Indeed, in those mutants, Sertoli cells overproliferate, lose expression of the male-specific Sox9 protein and acquire expression of female specific *Forkhead box L2* (*Foxl2*) and other granulosa markers. Germ cells fail to undergo radial migration, to reactivate mitosis, to enter meiosis and to survive beyond P10 [50]. In order to identify the function of *Dmrt1* in Sertoli and germ cells, Kim et al. have used conditional gene targeting. This approach revealed that Dmrt1 is required in Sertoli cells for their postnatal differentiation, for germ line maintenance and for meiotic progression. In germ cells, Dmrt1 is required for radial migration, for mitotic reactivation just after birth, and for survival beyond the first postnatal week. Thus, in mice, Dmrt1 is required autonomously in both cell lineages. Dmrt1 activity in Sertoli cells is also required non-cell autonomously to maintain the germ line [51].

A recent study has addressed the function of Dmrt1 in Sertoli cells in the postnatal testis. In mammals, sex is determined in the fetal gonad by the presence or absence of the Y chromosome gene *Sry*, which controls whether bipotential precursor cells differentiate into testicular Sertoli cells or ovarian granulosa cells. This pivotal decision in a single gonadal cell type ultimately controls sexual differentiation throughout the body. Sex determination can be viewed as a battle for primacy in the fetal gonad between a male genetic network in which Sry activates *Sox9* and a female network involving Wnt signaling and Foxl2, a female-specific transcription factor expressed in granulosa and theca cells. Loss of *Dmrt1*, even in adult Sertoli cells, activates ovary-specific genes such as *Foxl2* and causes the loss of male-promoting genes such as *Sox9*, and reprograms Sertoli cells into granulosa cells. In this environment, theca cells form, oestrogen is produced, and germ cells appear feminized [24, 25]. Conversely, loss of *Foxl2* in adult granulosa cells causes ectopic expression of *Dmrt1* and their reprogrammation into Sertoli cells [52]. Thus, Dmrt1 is required to prevent female reprogramming in the postnatal mammalian testis, and the sexual fate of the somatic gonad is postnatally controlled by the opposed activity of Dmrt1 and Foxl2.

Another function of Dmrt1 in male germ cells was revealed when Dmrt1 was deleted in postnatal undifferentiated spermatogonia. In mammals, meiosis begins at puberty, and sperm is produced throughout life. Spermatogenesis occurs in three phases: a mitotic proliferative phase involving spermatogonia stem/progenitor cells, two reductive divisions of meiosis, and then a postmeiotic phase of spermiogenesis. The switch from mitosis to meiosis requires retinoic acid (RA), which activates meiotic inducers, including Stra8 [53, 54]. Dmrt1 is detected in all mitotic spermatogonia, and its expression decreases with the onset of spermatogonial differentiation and disappears at the initiation of meiosis. Loss of Dmrt1 in undifferentiated spermatogonia causes them to precociously exit the spermatogonial program and enter meiosis. Dmrt1 appears to act in spermatogonia by suppressing RA via transcriptional repression of Stra8, and by promoting the production of the spermatogonial differentiation basic helix-loop-helix transcription factor Sohlh1 [55]. These data indicate that Dmrt1 is a transcriptional

Table 1 An outline of reported Dmrt factor embryonic expression in vertebrates

Factor	Species	Embryonic expression in non-gonadal tissue (in situ)	Expression in gonads	References
Dmrt1	Mouse	nd	Genital ridge prior to sexual differentiation. Expression becomes XY specific during gonadogenesis. Both in Sertoli and germ cells (mitotic spermatogonia).	[49, 55, 103]
	Chicken	nr	Genital ridge, higher in male. Becomes testis-specific after the onset of sexual differentiation.	[49, 103]
	Xenopus	nr	First detected in primordial gonad in both ZW and ZZ tadpoles in cells surrounding the PGCs. Expression is maintained and becomes higher in testis than ovary after metamorphosis.	[20, 71, 104]
(dm-w)	*Xenopus*	nr	Expression exclusively in primordial gonads of ZW tadpoles in cells surrounding the PGCs. Expression is not maintained in the ZW and ZZ gonads.	[71, 72]
	Platyfish	nd	Spermatogonia and Sertoli cells in adult testis.	[61, 105]
	Zebrafish	nr	Testis and ovary, higher in testis than ovary. Germ cells.	[106]
	Medaka	nr	No expression in gonads in embryos before stage 20 of development. Expressed in spermatogonium-supporting cells after testicular differentiations (20 days after hatching).	[62, 67, 93]
(Dmy)	Medaka	nr	Expressed during development from neurula stage in somatic cells surrounding germ cells exclusively in XY gonads. Expression maintained in adult testes.	[62, 63, 67]
Dmrt2	Mouse	Presomitic mesoderm and dermomyotome of somites.	Barely detectable expression, higher in testis than ovary during embryonic development.	[80, 83, 85, 86, 107]
	Chicken	Transient asymmetric expression in the chick Hensen's node, anterior presomitic mesoderm and dorsal compartment of somites.	nr	[84]
	Medaka	Somites.	Sertoli cells in adult testis. Early stage oocytes in adult ovaries.	[93]
	Zebrafish	Kupffer's vesicle in 3-somite stage embryos; presomitic mesoderm and newly formed somites in bud stage embryos (Dmrt2a). Somites and branchial (Dmrt2b).	Adult testis (Dmrt2b).	[80–83]
	Platyfish	Somites; branchial arches (Dmrt2a).	nd	[105]
Dmrt3	Mouse	Nasal placode, telencephalon, spinal cord interneurons.	Expression initially similar in testis and ovary; higher in testis after E13.5. Interstitial cells.	[87, 107]
	Chicken	Nasal placode, telencephalon, dorsal spinal cord interneurons, somites, müllerian ducts (higher in females than males).	nd	[87]
	Zebrafish	Nasal placode, anterior neural tube, dorsal spinal cord interneurons.	Undifferentiated gonads from 17 dpf. Adult testis (spermatogonia and spermatocytes) and ovary (oocytes).	[15]
	Medaka	Dorsal spinal cord interneurons.	Differentiating gonads (12–20 dph) and adult testis.	[93]
Dmrt4	Mouse	Ubiquitous, high expression in olfactory tissues.	Similar levels in testis and ovary (from E11.5).	[89, 107]
	Xenopus	Nasal placode, telencephalon, foregut, gall bladder.	Adult testis.	[88]
	Medaka	Olfactory system, telencephalon.	Differentiating gonads, adult testis and ovary.	[93, 108]
	Platifish	Olfactory placode, forebrain, branchial arches.	nd	[105]

Table 1 continued

Factor	Species	Embryonic expression in non-gonadal tissue (in situ)	Expression in gonads	References
Dmrt5	Mouse	Nasal placode, dorsal telencephalon, ventral forebrain/midbrain border at E9.5 extending later to the entire ventral midbrain, optic stalk, lateral head ectoderm, maxillary and mandibular processes, eyes, hypophysis.	Higher levels in ovary versus testis (E12.5 to E15.5).	[90, 91, 107]
	Xenopus	Nasal placode, dorsal telencephalon, ventral diencephalon.	nr	[9]
	Zebrafish	Nasal placode, dorsal and ventral telencephalon, ventral diencephalon.	Germ cells in adult testis and ovary.	[95, 109]
	Platifish	Forebrain, olfactory placode, midbrain, lens.	nd	[105]
Dmrt6	Mouse	nr	nd	[107]
Dmrt7	Mouse	nd	Higher level in ovary versus testis in embryonic and adult gonads. Postnatally, expression is male specific and restricted to spermatocytes and sperm.	[75, 76, 107]
Dmrt8	Mouse	nr	Higher level in testis than in ovary in embryonic gonads. Testis-specific expression restricted to Sertoli cells in the adult.	[14]

nr not reported, *nd* not detected

gatekeeper that controls the mitosis versus the meiosis decision in male germ cells.

The role of Dmrt1 in the mammalian fetal ovary has also been investigated. In females, meiosis begins in the fetus, and germ cells remain arrested in the diplotene stage of prophase I until after puberty. As in males, meiotic activation occurs under the influence of RA and the downstream meiosis inducer Stra8 [54, 56]. *Dmrt1* mutant germ cells were found to have severely reduced *Stra8* expression and to undergo abnormal meiotic prophase [23]. mRNA profiling and ChIP suggest that transcriptional activation of *Stra8* is the main function of Dmrt1 in the fetal ovary, and that this regulation is likely to be direct. Thus, Dmrt1 controls *Stra8* sex-specifically, activating it in the fetal ovary and repressing it in the adult testis.

Studies on the function of Dmrt1 in sexual development have also been conducted in non-mammalian vertebrates. In species with temperature-dependent sex determination such as turtles, lizards and alligators, *Dmrt1* expression was found to be higher in developing male gonads than in female ones, suggesting that it is involved in this process [57–59].

In the medaka, *Oryzias latipes*, which like mammals uses the XX/XY sex determination system, *Dmrt1* has undergone duplication. Gene duplication generating *Dmy* recently occured during evolution of the genus *Oryzias* as the gene is absent in other fishes, including other *Oryzias* species [60, 61]. One of the newly derived paralogs, called *Dm domain on Y*, also known as *Dmrt1bY* or *Dmy*, is located on the sex-determining region of the Y chromosome. *Dmy* is expressed exclusively in somatic cells of XY gonads in the early gonadal primordium before morphological sexual dimorphism is observed. *Dmy* is a master regulator of sex determination as it is both required and sufficient for male development [62–66]. The other paralog, *Dmrt1*, is expressed in spermatogonium-supporting cells, which is the same lineage of cells expressing *Dmy*, but after testis differentiation [67]. High temperature or steroid treatment induces *Dmrt1* expression in XX embryos and leads to XX sex-reversed testis [68, 69]. Conversely, in a *Dmrt1* mutant line, XY individuals developed into normal egg-laying females. The XY mutant gonads first developed into the normal testis type, but by day 10 after hatching, the gonads transdifferentiate into the ovary type [70]. These data suggest that *Dmrt1* in medaka is essential to maintain testis differentiation after *Dmy*-triggered male differentiation pathway.

Xenopus laevis uses the female heterogametic ZZ/ZW-type sex determining system. Similarly to medaka, a duplicated variant of *Dmrt1* residing on the female-specific W chromosome has been isolated (termed *dm-w*). While *Dmrt1* is expressed continuously in both ZZ and ZW developing gonads, *dm-w* is expressed exclusively in female ZW primordial gonads at sex determination. *dm-w* is an ovary-determining gene in *X. laevis* as exogenous dm-w causes developing ovotestes in ZZ tadpoles and dm-w knockdown in ZW individuals induces male development [71]. *Dm-w* appears to direct female sex by antagonizing the autosomal *Dmrt1* gene to determine a testis fate. *Dm-w* encodes a truncated Dmrt1 protein which has a DM domain but lacks more carboxy-terminal sequences. It is proposed that dm-w blocks Dmrt1 by dimerizing and antagonizing Dmrt1

Table 2 An overview of the phenotypes associated with loss or gain of function of chordate *Dmrt* genes

Factor	Species		Loss-of-function/gain-of-function phenotypes	References
Dmrt1	Mouse	KO	Defects in germ radial migration, reactivation of mitosis and survival. Failure of Sertoli cell differentiation. Teratoma formation in fetal germ cells. Failure to control the mitosis versus meiosis decision in male germ cells. Female reprogramming in the postnatal testis. Reduction of primordial follicles in the juvenile ovary.	[22–24, 50, 51, 56]
	Medaka	M	Male-to-female sex reversal after sex determination.	[70]
(Dmy)	Medaka	M	Female development in genetic males.	[62, 65]
		GOF	Male development in genetic females. Inhibition of primordial germ cell proliferation.	[66, 110]
		KD	Loss of proliferation inhibition in primordial germ cells.	[110]
(dm-w)	*Xenopus*	GOF	Ovarian cavities in gonads of ZZ tadpoles overexpressing dm-w.	[71, 72]
		KD	Male development in ZW individuals.	[72]
	Chicken	KD	Feminization of gonads of embryonic males.	[74]
Dmrt2	Mouse	KO	Embryonic somite patterning and myogenic defects.	[83, 85, 111]
		GOF	Increased myogenesis.	[86]
(*Dmrt2a/ Terra)*	Zebrafish	GOF	Increased apoptosis.	[80]
		KD	Randomization of left-sides specific genes and desynchronization of the segmentation clock.	[84]
Dmrt2b	Zebrafish	KD	Defects in somitogenesis and hedgehog signaling. Neural tube patterning defects. Randomization of left-side specific genes. Impairment of slow muscle development.	[81]
Dmrt3	Mouse	KO	Male sexual development abnormalities. Dental malocclusions.	[112]
		KO	Defects in spinal circuits involved in locomotion	[21]
	Horse	M	Defects in pattern locomotion in horses	[21]
	Xenopus	GOF	mDmrt3 promotes neurogenesis in caps.	[9]
Dmrt4	Mouse	KO	Females with polyovular follicles and male sexual development abnormalities.	[89]
	Xenopus	KD	Impaired neurogenesis in the olfactory placode.	[88]
		GOF	Promotes neurogenesis in caps.	[88]
Dmrt5	Mouse	KO	Reduced development of the caudomedial cerebral cortex.	[91, 92]
		GOF	Promotes dopamine neurons in ES cells.	[90]
		KD	Inhibition of ES cells differentiation towards a ventral medial mesencephalic cell fate.	[90]
	Xenopus	KD	Impaired neurogenesis in the olfactory placode.	[9]
		GOF	Promotes neurogenesis in caps.	[9]
	Zebrafish	M, KD	Defects in telencephalic neurogenesis.	[95]
(Dmrt1)	*Ciona*	M	Defects in anterior neural plate derivatives.	[100]
		KD	Impaired *Six1/2*, *Six3/6*, *Meis* and ZicL in the developing brain and *FoxC* in palps.	[99]
Dmrt7	Mouse	KO	Infertility with spermatogenic arrest in pachytene stage and sex chromatin defects.	[75, 76]

GOF gain of function, *KD* knockdown, *KO* knockout, *M* mutation

transcriptional activity [72]. As in medaka, this sex-determining role is a recent innovation. Indeed, a closely related species, *Silurana tropicalis*, lacks the *dm-w* gene [73].

Birds also use a female heterogametic sex ZZ/ZW system. Sex is supposed to be determined by the higher Z chromosome dosage in males, by the presence of a W chromosome in females, or possibly by a combination of both [35]. In all birds, *Dmrt1* is located on the Z chromosome. In embryos, it is expressed in the early bipotential gonad and shows higher expression levels in ZZ versus ZW embryos [49]. If *Dmrt1* activity is experimentally reduced, the gonads of genetically male (ZZ) embryos are feminized [74], demonstrating that Dmrt1 is required for testicular differentiation. Thus, in birds, Dmrt1 plays an important role in sex determination; the higher expression level in male (ZZ) embryos triggers the testis specification pathway whereas only a lower dose, as found in females, is compatible with ovarian development. Whether elevated *Dmrt1* expression is sufficient in ZW genital ridge to induce male development remains however to be tested.

Dmrt7 is required for male meiosis

Dmrt7 is present in placental mammals and marsupials but no ortholog has been reported in non-mammalian vertebrates. In mouse, *Dmrt7* is expressed in both male and female fetal gonads. In the ovary, *Dmrt7* expression is independent of the germ line, as, in XX c-kit mutants which lack germ cells, the level of its expression remains similar to that observed in wild-types. In adults, *Dmrt7* expression is male specific. It is predominantly detected in mid- to late-pachytene spermatocytes and the protein preferentially localizes to the XY body, a densely stained chromatin domain harboring sex chromosomes, essential for male meiotic progression. Consistent with this expression pattern, mice deficient in *Dmrt7* are infertile and most mutant cells show spermatogenic arrest in pachytene stage and abnormal cellular organization of Sertoli cells [75, 76]. The germ cell defects of *Dmrt7* mutants are not caused by aberrant Sertoli cell organization since animals with deletion of *Dmrt7* just in Sertoli cells have normal testis and spermatogenesis. *Dmrt7* mutant cells establish a normal XY body in mid-pachynema, but then have multiple epigenetic defects in the sex chromatin transition from pachynema to diplonema. This suggests that Dmrt7 plays a role in the control of the transition from meiotic sex chromosome inactivation to postmeiotic sex chromatin in males [76].

Dmrts are important during embryogenesis in non-gonadal tissues

Following their initial expression in the developing gonads, a subset of the *Dmrt* family members, including *Dmrt2*, *Dmrt3*, *Dmrt4* and *Dmrt5*, show differential expression in a limited number of non-gonadal tissues and organs. In most species, *Dmrt* genes have been detected in tissues such as the central nervous system, nasal placode or somites. Their expression pattern is often conserved across species, but there are also differences. For example, while *Dmrt3* is expressed in the neural tube and in the presomitic mesoderm in chick, it is only detected in the nervous system in fish and mouse suggesting its function has shifted during evolution (Table 1). Those four DM domain genes have been recently the subject of functional analysis in mouse, fish or *Xenopus*. Table 2 summarizes some of these gain- and loss-of-function studies and the resulting phenotypes. Here, we discuss these recent studies which show that Dmrts, like DSX, direct a variety of cell differentiation events and often function in the specification of progenitor cells. They also indicate that some of these Dmrt factors, as DSX in flies [33, 77–79], act by modulating signaling pathways, suggesting that this may be a common theme for DM domain proteins across species.

Dmrt2 is involved in establishing left–right asymmetry and somitogenesis

The first *Dmrt* gene suggested to have a role unrelated to sexual development was *Dmrt2*. It was first identified in zebrafish through a systematic search for genes with tissue-specific expression and was selected because of its somite and presomitic mesoderm-specific expression pattern. The identified gene, originally called *terra*, was found to play a role in somitogenesis based on the observation that its overexpression induces rapid apoptosis in the mesoderm [80]. A duplicated copy of the *Dmrt2* gene was later described in the zebrafish genome, and *Dmrt2a*/*terra* and *Dmrt2b* have been designated to distinguish them. Both genes are expressed during somitogenesis, but the fish-specific *Dmrt2b* is also expressed in branchial arches [81, 82]. In addition to the developing somites, *Dmrt2a* is transiently asymmetrically expressed in the zebrafish Kupffer's vesicle and in the equivalent structure in the chick, the Hensen's node, which suggest a left–right patterning function during development [83, 84]. Indeed, in zebrafish, using a morpholino-based approach, *Dmrt2a* was found to be required for left–right synchronization of the segmentation clock. It is also required for left–right patterning in the lateral plate mesoderm and thus the correct positioning of the internal organs on each side of the midline. As such, Dmrt2a is a key factor linking left–right patterning with bilateral synchronization of the segmentation clock in the mesoderm [84]. *Dmrt2b* morphants also display defects in heart and visceral organ asymmetry, and some lateral plate mesoderm markers expressed in the left side are randomized. *Dmrt2b* knockdown also leads to notable defects in somitogenesis

and reduces target gene expression of Hedgehog signaling, which results in significant impairment in slow muscle development. Dmrt2a cannot compensate for the loss of Dmrt2b and vice versa. These data indicate that functional divergence has occurred between the two duplicated genes, with *Dmrt2b* maintaining the common function for left–right establishment and contributing to a divergent function in somitogenesis through Hedgehog signaling [81].

In the mouse, *Dmrt2* is expressed in the presomitic mesoderm and is then confined to the dermomyotome, the dorsal epithelial domain of the developing somites containing muscle stem cells, but is absent from the node [83]. As in zebrafish, its targeted disruption leads to severe somite patterning defects, first visible at day E10.5. Both the dermomyotome and myotome fail to adopt a normal epithelial morphology. Accompanying these morphological defects, alterations in the expression of dermomyotomal and myotomal transcription factors such as *Pax3*, *Paraxis*, *Myf5*, *Myogenin*, *Mrf4* and *MyoD* were observed [85]. In agreement with the absence of its expression from the mouse node, *Dmrt2* homozygous mutants do not show left–right desynchronization of somite formation or defects in left–right asymmetric organ positioning. Thus, the role of *Dmrt2* in symmetric somite formation and in the regulation of the laterality pathway is not conserved during zebrafish and mouse embryonic development [83]. Whether this loss of *Dmrt2* function in left–right patterning in the mouse arise from mutations occurring in the enhancer responsible for the node expression or from the loss of a protein(s) necessary to activate specifically the node enhancer remains unknown. In a recent report, *Dmrt2* has been identified as a target of Pax3, a critical regulator of skeletal muscle stem cells. Furthermore, Dmrt2 was found to directly regulate early activation of the myogenic determination gene *Myf5*, required for the formation of the first skeletal muscle in the somites. Conditional overexpression of *Dmrt2* in Pax3-expressing cells in the somite confirms the role of this factor in the activation of *Myf5* [86]. Thus, a genetic network comprising *Pax3*/*Dmrt2*/*Myf5* operates in the muscle stem cells of the dermomyotome in the mouse embryo to orchestrate the onset of myogenesis.

Dmrt3, Dmrt4 and Dmrt5 play key roles in neurogenesis

Several studies have shown that members of the *Dmrt3–5* subfamily of DM genes are expressed in a restricted manner in the developing nervous system. All three genes are expressed in the developing telencephalon and olfactory placode in the mouse (Fig. 2), and this expression pattern in the developing CNS is conserved in most vertebrate embryos studied, including chick, mouse and zebrafish embryos (Fig. 3a–d) [87–92]. In addition, *Dmrt3* is also strongly expressed in the spinal cord in dorsal interneurons

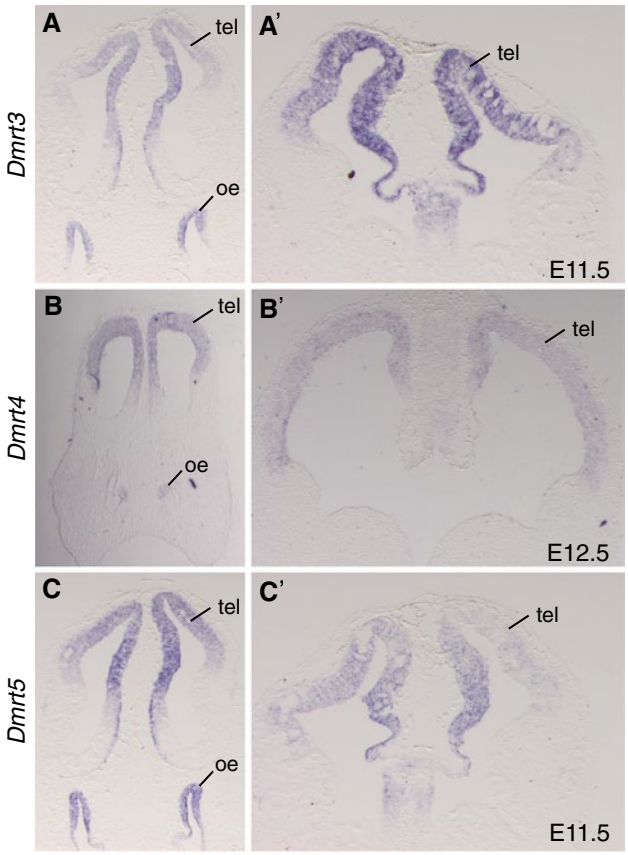


Fig. 2 In situ analysis of *Dmrt3*, *Dmrt4* and *Dmrt5* expression in the head of mouse embryos. Coronal sections at the indicated stages in the nasal region (**a**–**c**) and at the level of the anterior telencephalon (**a′**–**c′**) are shown. All three genes are expressed in a graded manner in the developing telencephalon and in the olfactory epithelium. *Oe* olfactory epithelium, *tel* telencephalon

[15, 87, 93] and *Dmrt5* in the ventral medial mesencephalon (Fig. 3a) [90, 91]. The functional relevance of these expression patterns has recently been investigated for *Dmrt4* and *Dmrt5* in *Xenopus* olfactory placode and zebrafish telencephalon neurogenesis and for *Dmrt3* in mouse spinal cord neuronal specification (Fig. 4).

In *Xenopus*, *Dmrt4* and *Dmrt5* are coexpressed early in the anterior neural ridge (ANR) and then become progressively restricted to the dorsal telencephalon and the olfactory epithelium. Both genes are positively regulated by neural inducers and negatively by proneural factors. They are also activated by the combined action of the transcription factor Otx2, broadly transcribed in the head ectoderm, and of Notch signaling, activated in the ANR. Knockdown of Dmrt4 or Dmrt5 impairs neurogenesis in the embryonic olfactory system and in neuralized animal caps. Conversely, their overexpression promotes neuronal differentiation in animal caps as visualized using markers such as the bHLH transcription factors *Ngnr-1*, *Ebf2* or *Ath5*, a property that requires the C-terminal DMA domain and downstream

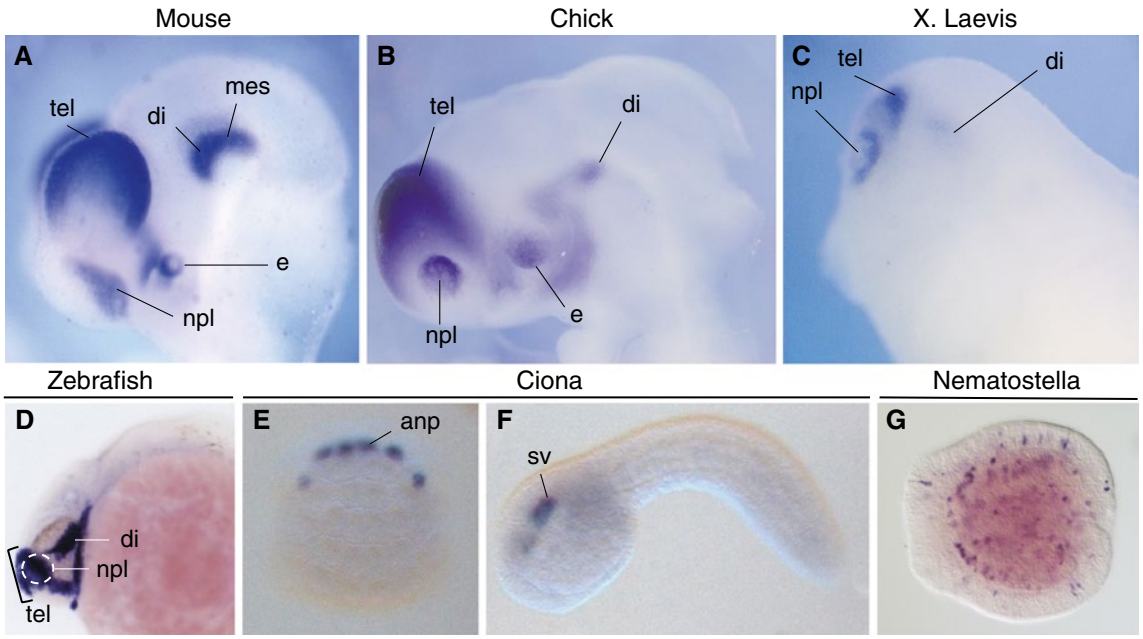


Fig. 3 Whole-mount in situ analysis of *Dmrt5* expression in the mouse, chick, frog and fish embryos and of related genes in ascidian and cnidarian. **a–d** In mouse (stage E10.5), chick (stage 18), *Xenopus laevis* (stage 28) and zebrafish (24 hpf) embryos, *Dmrt5* is strongly expressed in the nasal placode and dorsal telencephalon. It is also highly transcribed in the ventral diencephalon in chick, frog and zebrafish embryos and in the mouse in the ventral diencephalon and mesencephalon. **e**, **f** In *Ciona* 64-cell stage embryos (**e**), *Dmrt1* that is related to *Dmrt4/5*, is expressed in progenitors of the anterior neural plate and later at tailbud stage (**f**) is restricted to the anterior brain. (Images from Ghost database, http://ghost.zool.kyoto-u.ac.jp/SearchGenomekh.html). **g** In *Nematostella vectensis*, *NvDmrtB* that is related to *Dmrt4/5* is expressed in scattered cells in both the ectodermal and endodermal layers in late planula embryos. The strongest staining is most prominent at that stage in the endoderm, correlating with the onset of neurogenesis. In (**a–d**), lateral views of the head of the embryos are shown with anterior to the *left*. In (**e**), the anterior side of the embryos is to the *top*. In (**f**), lateral view of a tailbud stage embryo is shown. In (**g**), the blastopore is to the *right*. *Anp* anterior neural plate progenitors, *Di* diencephalon, *e* eye, *mes* mesencephalon, *npl* nasal placode, *sv* sensory vesicle, *tel* telencephalon

sequences. In animal caps, the Noggin mediated induction of *Ngnr-1* and *Ebf2* that is affected by the depletion of Dmrt5 can be rescued by *Dmrt4* overexpression. Conversely, the inhibition of *Ngnr-1* and *Ebf2* in the context of Dmrt4 depleted explants can be rescued by *Dmrt5* overexpression [9, 88]. Together, these data indicate that Dmrt4 and Dmrt5 have overlapping functions upstream of proneural factors during olfactory placode neurogenesis.

In the mouse, *Dmrt3*, *Dmrt4* and *Dmrt5* are strongly expressed in the developing olfactory epithelium but it is not known whether they play a role in its formation [87, 89, 91]. *Dmrt4*-deficient mice have been generated. Those mice are viable and fertile but have polyovular follicles, suggesting a role in folliculogenesis. Interestingly, 25 % of the mutant males exhibited copulatory behavior towards other males. As olfaction and sexual behavior are strongly linked in mice, this suggest possible olfactory function defects [94]. Nevertheless, *Dmrt4*-deficient mice have a histologically normal olfactory epithelium and general olfaction [89]. In contrast, the olfactory epithelium is reduced in *Dmrt3* and *Dmrt5* knockout (KO) mice and is almost completely absent in *Dmrt3*:*Dmrt5* double KO (Saulnier et al., unpublished data). The lack of phenotype in *Dmrt4* mutants is thus likely to be due to the presence of *Dmrt3* and *Dmrt5*. Together, these observations indicate that *Dmrt3-5* may have overlapping function in vertebrate olfactory placode development, which remains to be further investigated.

In zebrafish, a *Dmrt5* mutant was isolated that shows defects in telencephalic neurogenesis. Expression of *Neurog1* and other telencephalic marker genes such as *Foxg1* and *Emx3* were downregulated, while in contrast, *Her6*, a *Hes*-related gene that encodes a negative regulator of *Neurog1*, was expanded. Knockdown of Her6 rescues *Neurog1* expression in the *Dmrt5*$^{-/-}$ telencephalon, suggesting that Dmrt5 regulates *Neurog1* expression by repressing *Her6* [95]. Such a mechanism has been previously reported for MAB-3 in the specification of sex-specific neurons in *C. elegans* [26]. So, Dmrt5 regulates neurogenesis in the zebrafish posterior-dorsal telencephalon. Whether the other *Dmrt* genes expressed in the developing telencephalon also play a role in neurogenesis, and whether this function in neurogenesis is conserved among vertebrates, remains to be investigated.

A *Dmrt4/5*-related gene has been recently identified in the sea anemone *N. vectensis* (designated *NvDmrtB*), a model

system from the sister group of bilaterians, the cnidarians. In *Nematostella*, *NvDmrtB* is expressed in scattered neural cells in both the ectodermal and endodermal layers (Fig. 3g). In *Xenopus*, its overexpression induces neurogenesis in animal cap explants. Conversely, knockdown of NvDmrtB in *Nematostella* reduces the number of Elav-1 positive neurons [9]. These results suggest that Dmrt's ability to control neurogenesis derives from an ancestral function already present in the last common ancestor of cnidarians and bilaterians. Further analyses of *Dmrt* genes in basally branching animals, including sponges, will be required to define the ancestral functions of *Dmrt* genes.

A very recent study has shown that a *Dmrt3* nonsense mutation has a major effect on the pattern of locomotion in horses. The phenotype indicates that Dmrt3^{+} spinal cord neurons have a critical role for left–right coordination and for coordinating the movement of the fore- and hind-legs. *Dmrt3* is expressed in cells originating from dI6 progenitors that develop into inhibitory interneurons projecting ipsi- and contralaterally. Examination of wild-type and *Dmrt3* null mice demonstrated that it takes part in neuronal specification within this subdivision, and that it is critical for spinal circuit function. The mutation leads to a truncated Dmrt3 protein retaining the DM and DMA domains but lacking the downstream sequences, which highlight their importance for its activity [21].

Dmrt5 is required for anterior neural tissue development

In the mouse, three recent studies, two using KO mice and the third using embryonic stem (ES) cells, have provided insights into the function of Dmrt5 in the development of the telencephalon and mesencephalon, respectively (Fig. 4).

Regionalization of the telencephalon is initiated by morphogens secreted from localized inductive centers. The two major patterning centers that are the most directly implicated in telencephalon patterning are the ANR located at the rostral pole of the telencephalon and the roof plate and cortical hem (CH) region at the dorsal caudal midline and immediate adjacent territories. The ANR secretes FGFs and the CH produces a variety of Wnt and Bmp ligands critical for hippocampus development. These signals establish graded expression of genes encoding transcription factors that are crucial for the regionalization of the telencephalon and subsequent arealization of the cerebral cortex in cortical progenitors. Among them are Emx2, promoting a caudomedial fate, and Pax6, promoting a rostrolateral identity [96, 97]. In the mouse, similarly to *Emx2*, *Dmrt5* is detected in cortical progenitors in a high caudomedial to low rostrolateral gradient and is dependent on Wnt signaling [91, 92, 98]. With respect to the other transcription factors playing a crucial role in cortical development and patterning, it has been shown that *Dmrt5* is dependent on the early zinc finger

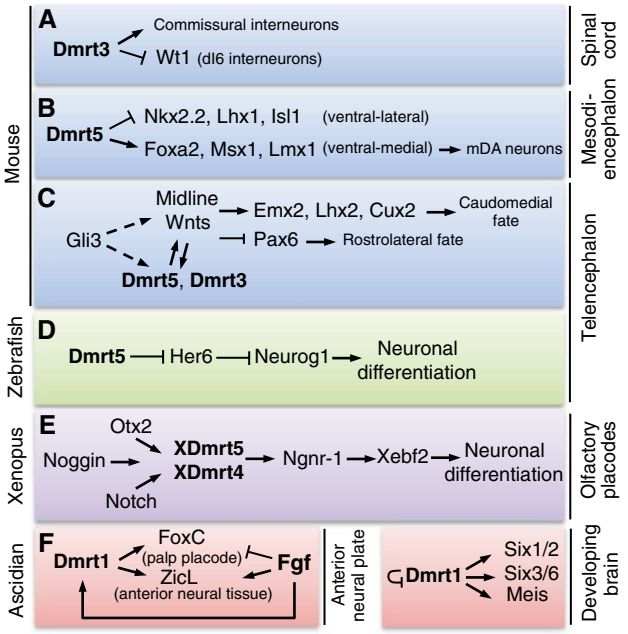


Fig. 4 Schematic representation of the involvement of Dmrts in neural development. **a** In the mouse spinal cord, loss of *Dmrt3* increases the number of Wt1^{+} neurons and results in fewer commissural interneurons. **b** In mouse ES cells, Dmrt5 induces ventral-medial midbrain progenitor markers and inhibits ventral-lateral ones, suggesting that in vivo, in the ventral-medial mesencephalic neuroepithelium, it enhances the acquisition of a mDA neuronal fate. **c** In the mouse telencephalon, *Dmrt5* and *Dmrt3* are targets for Wnt signaling and are dependent on Gli3. Direct or indirect action of Gli3 on *Wnts* and on *Dmrt5* and *Dmrt3*, through regulation of the Wnt signaling pathway, is indicated by dashed lines. Dmrt5 in turn is required for *Wnt* and *Bmp* expression in the dorsal midline signaling center and the expression of their downstream targets, that specify a caudomedial fate. *Pax6* is upregulated in *Dmrt5* mutants, presumably through its negative regulation by Wnts and Emx2. **d** In zebrafish, Dmrt5 is required for neurogenesis in the telencephalon, possibly by repressing Her6. **e** In the frog, Dmrt5 and Dmrt4 act downstream of neural induction, Otx2 and Notch and upstream of Neurogenin to control olfactory placode neurogenesis. **f** In *Ciona*, Fgf signals play a crucial role in nervous system development, activating several neural genes including *Dmrt1*. It also controls cell fate choice between the palp placodes and anterior central nervous system, which express *FoxC* and *ZicL*, respectively. *FoxC* and *ZicL* expressing cells derive from common progenitors that express Dmrt1 and both genes require Dmrt1 for their activation. Dmrt1 plays also a role in the promotion of *Six1/2*, *Six3/6* and *Meis* in the developing brain and negatively regulates its expression

transcription factor Gli3. In contrast, *Emx2* is not required for *Dmrt5* expression. *Pax6*, expressed in a complementary manner to *Dmrt5*, may antagonize its expression. In *Dmrt5* null mutants, the caudomedial cortex, including the hippocampus, is strongly reduced. Wnt and Bmp signaling in the embryonic dorsomedial telencephalon and the downstream-dependent transcription factors such as Emx2 are reduced. Pax6, which is inhibited by midline signals, is upregulated [91, 92]. In contrast, conditional ablation of

Dmrt5 at neurogenic stages using the *Nestin-Cre* transgenic line only causes a slight reduction in telencephalon size [92]. Thus, Dmrt5 is a novel Wnt-dependent "early master" regulator of initial regional patterning of the cerebral cortex. It is likely to function, at least in part, by promoting dorsal midline signaling center formation and thereby helping to establish the graded expression of the other transcription regulators of cortical identity. Whether Dmrt3 and Dmrt4 functions overlap with that of Dmrt5 in cortical patterning, and whether they directly or indirectly regulate *Wnt* and *Emx* gene expression, are two important questions still to be addressed.

Ascidian belongs to the urochordates, which represent the closest living relatives of the vertebrates. A gene related to *Dmrt4/5* with a DMA domain has been identified in the ascidian *Ciona savigny* (designated *Dmrt1*). In ascidians, the CNS develops via neurulation of cells of the neural plate, forming a simple brain called the sensory vesicle and a caudal nerve cord. The anterior neural plate produces placodal derivatives, such as the adhesive palps and stomodeum, and the anterior portion of the brain, called the sensory vesicle. *Dmrt1* is expressed from the 64-cell stage in progenitors of the anterior neural plate and is later restricted to the anterior part of the sensory vesicle (Fig. 3e, f). A null mutation in the *Dmrt1* gene as well as knockdown experiments have shown that *Dmrt1* is required for the development of the palps and oral siphon (mouth) that derive from the stomodeum. It also leads to extensive disruption of sensory structures, such as light-sensitive ocellus, in the sensory vesicle [99–101]. Furthermore, knockdown experiments have shown that *Dmrt1* is activated by FGF signals and is required for the expression of *FoxC* and *ZicL* that marks the palp placodes and anterior neural tissue, respectively. They also show that Dmrt1 promotes *Six1/2*, *Six3/6* and *meis* in the developing brain and negatively regulates its own expression [99]. In *Ciona*, as in vertebrates, *Dmrt* genes thus mark anterior neural regions, including placodes and anterior neural tissue, and are required for their development (Fig. 4).

The ventral-medial caudal diencephalon and mesencephalon contain dopaminergic neurons that are essential for the control of voluntary movements and the regulation of emotion, and are severely affected in neurodegenerative diseases such as Parkinson's disease. In response to local inductive signals (Shh, Fgf8, Wnt1), transcription factors are expressed at specific dorsal and ventral positions in the mesodiencephalon inducing distinct cell fates, including in the ventral-most progenitors a midbrain dopaminergic cell fate. As in the developing telencephalon, expression of *Dmrt5* in the ventral-medial mesencephalon is restricted to progenitors that give rise to dopamine neurons. In embryonic stem (ES) cells, overexpression of *Dmrt5* induces a ventral-medial progenitor phenotype and inhibits ventral-lateral mesencephalic markers. Conversely, knockdown compromises ES cell differentiation toward a ventral-medial cell fate [90]. Thus, Dmrt5 promotes midbrain dopaminergic (mDA) identity in ES cells by enforcing a ventral progenitor fate. Whether Dmrt5 controls in vivo ventral-medial mesencephalic cell fate remains to be demonstrated.

Conclusion

Besides their role in sexual development, Dmrt factors have clearly emerged as important regulators of vertebrate development. Recent findings indicate that members of the group A function as critical regulators of the development of the nervous system, functioning both in neurogenesis and patterning of the developing neural tissue, and that this ability to control neural development is an ancestral function. A better understanding of when and where these Dmrt factors binds to the genome is needed to provide insight into the molecular mechanisms employed by these factors. Elucidating the role of the conserved DMA domain will also be central to understand how Dmrt factors coordinate developmental processes, functioning as activators or repressors depending on the cellular context or locus. Future studies should also determine whether they play any cell-autonomous roles in sexual differentiation of non-gonadal tissues and whether their deregulation is associated with some human neural diseases.

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