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Sex and the singular DM domain: insights into sexual regulation, evolution and plasticity

Clinton K. Matson and David Zarkower

Department of Genetics, Cell Biology, and Development, University of Minnesota, 6-160 Jackson Hall, 321 Church Street SE, Minneapolis, Minnesota 55455, USA.

Abstract

Most animals reproduce sexually, but the genetic and molecular mechanisms that determine the eventual sex of each embryo vary remarkably. DM domain genes, which are related to the insect gene *doublesex*, are integral to sexual development and its evolution in many metazoans. Recent studies of DM domain genes reveal mechanisms by which new sexual dimorphisms have evolved in invertebrates and show that one gene, *Dmrt1*, was central to multiple evolutionary transitions between sex-determining mechanisms in vertebrates. In addition, *Dmrt1* coordinates a surprising array of distinct cell fate decisions in the mammalian gonad and even guards against transdifferentiation of male cells into female cells in the adult testis.

Sexual reproduction is ancient and nearly universal among animals. Despite the antiquity and ubiquity of sex, the upstream mechanisms that control sex determination and sexual differentiation in animals — which ultimately lead to differences in morphological, physiological and behavioural traits — are highly diverse and can include a variety of genetic or environmental signals (FIG. 1). These signals include sex-specific chromosomes (for example, XX versus XY), temperature, social cues and the development of fertilized versus unfertilized eggs (in social insects such as bees and wasps).

Detailed studies of sex determination and sexual differentiation have been performed in model organisms — mainly nematodes, insects and vertebrates — and have revealed some general regulatory features. The upstream sex determination pathways can use varied molecular mechanisms, including alternative splicing, translational regulation and extracellular signalling^{1,2}. By contrast, downstream control at the interface between sex determination and sexual differentiation tends to rely more heavily on transcriptional regulators. As a consequence, *cis*-regulatory elements control many sexually dimorphic features, and the functional interaction between sex-specific transcription factors and spatially or temporally specific transcription factors is central to the development and evolution of sexually dimorphic features³.

In the past decade, it has become clear that although the upstream sex determining signals are diverse and can be fast changing^{4–8}, they often act through more ancient downstream regulatory hierarchies that involve members of a specific family of transcriptional regulators — those that contain the DM domain DNA binding motif (BOX 1). The DM domain was initially recognized in a comparison between *doublesex* (*dsx*) from the fruitfly *Drosophila melanogaster* and *male abnormal 3* (*mab-3*) from the nematode worm *Caenorhabditis elegans*⁹. The discovery of functional and molecular similarities between *dsx* and *mab-3*

Correspondence to D.Z. zarko001@umn.edu.

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immediately suggested that DM domain genes might also regulate sex in vertebrates, and this soon led to the identification of *Dsx*- and *mab-3*-related transcription factor 1 (*DMRT1*)⁹. This was followed by the discovery of other Dmrt genes: some that are involved in sexual dimorphism and some that apparently are not^{10–12}. More recent studies have linked DM domain genes to sexual differentiation in many other species, providing valuable entry points for understanding the control of sexual differentiation in diverse animals.

Different species or taxa, and even related species with similar body plans, can exhibit startlingly different sexual dimorphisms — consider the tail of the peacock or the horns of the stag beetle¹³. It might seem likely that these different characters would be controlled by different sexual regulatory genes, and this is doubtless true in some cases. However, conserved DM domain genes have been found to control development of many different sexually dimorphic features in highly diverged species, suggesting that at least some of the time it is the deployment of these regulators that differs, rather than the regulators themselves. Close comparisons of DM domain function between species, particularly in insects, are confirming this view and are beginning to reveal how new sex-determining mechanisms (SDMs) can arise and how sexually dimorphic traits can be formed or modified. Not all of the lessons have been evolutionary: studies of DM domain genes are also illuminating how sexual regulation intersects with spatial and temporal regulation during development and providing surprising insights into sexual differentiation in mammals. In the case of DM domain genes, it seems that an ancient regulatory module has adapted to respond to different sex-determining signals and to control varied downstream effectors, some conserved and some not, to achieve sexual differentiation. This Review summarizes the roles of DM domain genes in metazoan sexual differentiation, focusing particularly on flies, worms and mice, in order to examine some of the major insights that have come from the study of this gene family.

Invertebrate sexual dimorphism

DM domain genes have been studied in particular depth in two model invertebrates — *D. melanogaster* and *C. elegans* — and we consider their functions in these species and related species before examining their roles in vertebrates. A key biological difference between vertebrates and invertebrates, at least in the species that have been studied to date, concerns where in the body sex is determined. In mammals and probably in other vertebrates, sex is determined in the gonads, and sexual differentiation in non-gonadal tissues and organs is initiated by the action of gonadal sex hormones. By contrast, in worms and in flies, although secreted signalling molecules are certainly important in generating sexual dimorphism, both upstream and downstream components of sex-determining pathways directly act on cells and tissues throughout the body. The more spatially distributed action of sex-determining genes in flies and worms tends to blur the distinction between sex determination and sexual differentiation, but it is still possible to distinguish between upstream ‘global’ regulators of sex and downstream effector genes that mediate sexual differentiation more directly by controlling the genes that carry out this process. So far, DM domain genes in invertebrates appear to function exclusively in this latter capacity, acting downstream of the primary sex determination decision to drive sex-specific development in varied tissues and cell types. This is also true in vertebrates, although, as discussed below, DM domain genes have periodically risen to ‘capture’ the top position in sex determination during vertebrate evolution.

Flies

Insects have multiple DM domain genes, of which only *dsx* has been shown to regulate sexual dimorphism. The *dsx* gene is a downstream regulator of sexual dimorphism in a wide range of insects, linking sexual differentiation to varied upstream trigger mechanisms¹⁴.

Sex-specific alternative splicing of *dsx* mediated by *transformer* (*tra*) and *tra2* is widely conserved in insects with a variety of sex determination systems, but *dsx* functions downstream of divergent global regulators such as *Sex lethal* (*Sxl*) in flies and *complementary sex determiner* (*csd*) in honeybees^{7,14–17}. So far, functional regulation of DM domain genes by alternative splicing has only been shown in insects.

In *D. melanogaster*, *dsx* controls virtually all somatic sexual dimorphism. Exceptions are some aspects of muscle and nervous system dimorphism, although this could be accounted for by the complex and non-ubiquitous expression pattern of *dsx*, as discussed below¹⁸. In flies, *dsx* acts in parallel with other downstream effectors of sexual differentiation, most notably *fruitless* (*fru*) and *dissatisfaction* (*dsf*). A few similarities can be seen between the functions of *dsx* in flies and *DMRT1* in vertebrates. Although *dsx* is widely expressed later in development, in embryos, it is specific to cells of the somatic gonadal primordium (SGP), much like *DMRT1* in vertebrates, as well as to a cluster of mesogermal SGP (msSGP) cells that only survive and join the gonad in males in response to male *dsx* activity^{19,20}. In another parallel with vertebrate male gonad development, the fly homologue of the testis-determining sex-determining region of chromosome Y (SRY) box 9 (*Sox9*) transcription factor, *Sox100B*, is expressed in the msSGP cells and is required specifically in males for gonadal differentiation^{20,21}.

Worms

C. elegans has 11 DM domain genes, of which three — *male abnormal 3* (*mab-3*), *mab-23* and *DM domain 3* (*dmd-3*) — are known to regulate sexual development. These genes appear to have more limited functions than those of *dsx* and function only in males, mainly in the male tail. The three DM domain genes have distinct and overlapping roles in tail development, controlling morphogenesis and differentiation of copulatory structures, such as the sensory rays, spicules and mating muscles²². Although these three DM domain genes have separate functions in male development, they also have functional overlaps. For example, *dmd-3* and *mab-3* act together to activate the fusogen *epithelial fusion failure 1* (*eff-1*): this promotes male-specific cell fusion and regulates unknown targets that stimulate tail-tip retraction during morphogenesis²³. In addition, *dmd-3* acts with *mab-23* to specify neuronal subtypes and muscle cell development in a neural circuit in the male tail that is essential for mating²⁴. *mab-3* also functions outside the tail, repressing yolk gene (*vitellogenin*) transcription in the intestine²⁵ and mediating mate recognition by males, mostly likely in the head²⁶.

In nematodes, DM domain genes work in conjunction with general developmental regulators: they modulate the activity of homeobox (Hox) genes and other conserved regulators and intersect with signalling pathways. Ray neurogenesis requires Hox-gene-mediated activation of the conserved proneural basic helix–loop–helix (bHLH) gene *lineage defective 32* (*lin-32*). This activation requires MAB-3, which blocks transcription of the antineural bHLH gene *regulator of fusion 1* (*ref-1*), a repressor of *lin-32*, in ray precursor cells²⁷. The *mab-23* gene acts later in ray development, patterning ray neuron subtypes in cooperation with the *Abdominal B* (*AdbB*) homologue *egg-laying defective 5* (*egl-5*) and a transforming growth factor- β (TGF β)-related signalling pathway²². A recent genome-wide screen for regulators of tail morphogenesis has elaborated on the interactions of DM domain genes with general regulators in the worm. This study suggests that a complex regulatory pathway converges on *mab-3* and *dmd-3* and that the activity of this pathway integrates sexual regulation with spatial and temporal regulation via surprisingly numerous and varied regulatory inputs and outputs²⁸.

Other invertebrates

DM domain genes are broadly implicated in metazoan sexual regulation. Expression of a DM domain gene is associated with reproduction in a coral²⁹. Recently, DM domain genes have been found to promote male gonadal differentiation in the flatworm *Schmidtea mediterranea* (T. Chong and P. Newmark, unpublished data) and to direct male versus female development of dimorphic structures, including the somatic gonad, germline, first antenna and copulatory thoracic hook in *Daphnia magna*³⁰, which is a crustacean with environmental sex determination. The involvement of DM domain genes in these species indicates that genes of this family are likely to control sexual differentiation in all of the major animal groups.

Interaction with general regulators

Sexually dimorphic features can come and go rapidly during evolution as a result of powerful sexual selection. As described above, studies in worms have helped to show how sexual regulation intersects with other forms of developmental regulation (such as spatial and temporal control and cell-type specification) to direct sexual dimorphism properly. Similar processes take place in the fly. In the fly genital disc, for example, DSX modulates *wingless* and TGF β signalling to drive sex-specific expression of the developmental regulator *dachshund* (*dac*)^{31,32}. It also controls male-specific fibroblast growth factor (FGF) signalling, which is required to recruit mesodermal cells in the male genital disc during larval development³³. Furthermore, *dsx* functionally interacts with *Hox* genes to establish sexual dimorphism in tissues throughout the body. This is clearly illustrated by two examples that involve abdominal patterning. In the posterior abdominal cuticle, females have weaker pigmentation than males owing to the female-specific expression of the *bric a brac 1* (*bab1*) and *bab2* transcription factor genes. Expression of *bab1* and *bab2* in the posterior abdomen is jointly regulated by *dsx*, which provides sex specificity, and by *AdbB*, which provides spatial control^{34,35}. The *dsx* gene also functionally interacts with *AdbB* to control sex-specific abdominal segmentation: DSX^M and ADBB jointly repress the Wnt ligand *wingless* in the terminal male abdominal segment, resulting in the male-specific loss of this segment during pupation³⁶. By interacting with these general developmental regulators, *dsx* integrates temporal, spatial and sexual inputs and produces varied sexually dimorphic structures in flies, similar to the way that *mab-3* and *dmd-3* integrate temporal and spatial regulation in the worm. A minor distinction between the two species is that sexual regulatory functions are distributed among several DM domain genes in worms rather than being concentrated in one gene, as in flies. Identification of additional downstream targets (for example, REF. 37) will further clarify the degree of conservation in sexual differentiation mechanisms controlled by DM domain genes. It seems likely that functional interaction between DM domain genes, conserved Hox genes and signalling pathways will prove to be a general theme in metazoan sexual differentiation.

Evolution of novel dimorphisms

Studies in *D. melanogaster* have been particularly helpful in revealing the mechanisms by which new sexual dimorphisms can arise during evolution. Until recently, it was assumed that the specific effects of DSX in different target cell types might solely be due to functional interactions with other more spatially and temporally restricted transcription factors. However, this is not the case. Expression of DSX itself is remarkably complex and, as a result, whereas most or all cells ‘have’ a sex in the sense that they have sex-specific activity of the upstream regulator *Sxl*, only a subset of cells are able to ‘know’ their sex because they express *dsx* and can respond directly to the alternative splicing cascade that is controlled by *Sxl*^{18,19,38}. Recent work has shown that evolution of new sexually dimorphic traits in the fly has exploited both the complexity of *dsx* expression and the ability of *dsx* to

control downstream target genes in collaboration with developmental regulators such as *AbdB* (BOX 2).

Vertebrate sex determination

Broad conservation

As described earlier, in mammals and probably in other vertebrates, the primary sex determination decision occurs first in the gonad, and then gonadal hormones direct the rest of the body into a male or a female developmental and physiological mode (secondary sex determination). Accordingly, genes involved in mammalian sex determination are expressed in the somatic cells of the embryonic gonad before the onset of sexual differentiation. Consistent with such a role, *Dmrt1* is expressed in the gonadal primordium (the genital ridge) in mammals and, indeed, in all other vertebrates examined so far^{39–41}. In humans, *DMRT1* is localized to a short chromosomal region on chromosome 9 (9p24.3) whose hemizygosity is associated with testicular dysgenesis and, in some cases, with the male-to-female sex reversal of XY individuals^{9,42,43} (BOX 3).

In mice, *Dmrt1* is not required for primary sex determination — XY mutants are born as males with testes⁴⁴ — and hence it remains unclear whether *Dmrt1* regulates primary male sex determination in mammals. However, studies in non-mammalian vertebrates, which are described in the following section, have provided important insights into the evolution of SDMs by revealing how *Dmrt1* or a close paralogue has ‘captured’ control of sex determination in fish, birds and frogs, in each case by a different means (FIG. 2).

Fish

Fish have a spectacular diversity of sex-determining systems and reproductive lifestyles⁴¹. In all fish examined, *Dmrt1* is expressed in the developing male gonad, including in sequential hermaphrodites undergoing ovary-to-testis transition⁴⁵. In at least one case, a *Dmrt1* gene has taken over sex determination: in the teleost fish medaka (*Oryzias latipes*, XX/XY sex determination), a gene duplication of the autosomal *Dmrt1a* generated a new Y chromosome on which the new *Dmrt1* gene, called *DM domain on Y* (*Dmy*; also known as *Dmrt1y*), acts as a male-linked dominant ‘master regulator’ of sex determination and is both necessary and sufficient to trigger male development^{46,47}, much like *SRY* in mammals. The discovery of *Dmy* beautifully illustrates how a conserved downstream sex regulator can acquire control of the sex determination hierarchy through a straightforward mutational event. *Dmrt1* may also function in sex determination in other fish: overexpression of the Nile tilapia *Dmrt1* gene causes partial to complete XX female-to-male sex reversal in these fish⁴⁸ and, in zebrafish, genome-wide linkage analysis has implicated a locus containing *Dmrt1* as a major determinant of sex⁴⁹.

Birds

Dmrt1 appears to control avian sex determination by a different genetic mechanism. Birds have ZZ/ZW genetic sex determination, and sex is 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. *Dmrt1* is Z-chromosome-linked in all birds, including ratites, such as ostriches and emus, and it shows elevated expression in ZZ versus ZW genital ridges before gonadal differentiation^{39,40,50}. Depletion of *Dmrt1* in chick embryos by RNA interference (RNAi) caused the gonads of ZZ embryos to develop with a feminized morphology, demonstrating that *Dmrt1* is essential for testicular differentiation and suggesting the possibility that the higher dose of this gene in males determines avian sex⁵¹.

The role of *Dmrt1* in avian sex determination remains ambiguous, however, for two reasons. First, although *Dmrt1* is necessary for male gonadal differentiation, it is not known whether elevated *Dmrt1* expression is sufficient for male development (that is, whether *Dmrt1* overexpression in the ZW genital ridge can fully induce male development). Second, the phenotype of avian gynandromorphs raises questions about the relative importance of the gonad in avian sex determination. In chicken and zebra finch bilateral gynandromorphs (in this case, animals with a majority of ZZ cells on one side of the body and ZW cells on the other), sexually dimorphic features including plumage, combs, spurs, musculature and neural song circuits develop with bilateral asymmetry^{52,53}. This strong genetic autonomy of sex severely undercuts the traditional view that vertebrate sexual dimorphism is mainly under non-autonomous hormonal control by the gonad. Clearly, in birds, sex chromosome composition also has a crucial cell-autonomous function in determining the sex of non-gonadal tissues and organs. *Dmrt1* cannot be directly involved in this extragonadal process because its expression is limited to urogenital tissues. Sex hormones do have a role in avian sex determination: oestrogen-treated ZZ embryos develop as females, whereas oestrogen-depleted ZW embryos develop as males⁵⁴. One possibility, therefore, is that differential dosage of *Dmrt1* controls male versus female development in the gonad, whereas other genetic factors, which are either Z- or W-linked, control the ability of the non-gonadal cells to respond to gonadal sex hormones that are regulated by *Dmrt1*. In this regard, avian sex determination may be formally analogous to that of flies, in which sex determination also is bipartite: it requires a universal signal from the sex chromosomes to set the state of *Sx* but also requires expression of *dsx* in specific cells to make them competent to respond to the *Sx*-dependent regulatory cascade and undergo sexual differentiation¹⁸.

Amphibians

Ito and colleagues⁵⁵ recently found that a duplicated variant of *Dmrt1* resides on the W chromosome of the frog *Xenopus laevis* (which also has ZZ/ZW genetic sex determination) and serves as an ovarian determinant. The *X. laevis* sex chromosomes are not homologous to those of birds and, unlike in birds, frogs also have an autosomal *dmrt1* gene. The W-linked determinant, *dmw*, encodes a truncated protein that has a DM domain but that lacks more carboxy-terminal sequences. The *dmw* protein can heterodimerize with *dmrt1* and is proposed to act as a dominant-negative inhibitor of masculinization, blocking testis differentiation and thus allowing ovarian development. It therefore appears that in medaka and *X. laevis*, mutational events involving *dmrt1*, one gain-of-function and one loss-of-function, occurred coincidentally with a transition between SDMs and may have been a driving force in these transitions. Phylogenetic studies show that these events occurred recently: ~10 million years ago in the case of the fish⁵⁶ and subsequent to the separation of *X. laevis* and *Xenopus tropicalis* in the case of the frog.

Other vertebrates

Studies in reptiles with temperature-dependent sex determination^{40,57,58} suggest that *Dmrt1* is likely to function in these processes. From the widespread involvement of *Dmrt1* in vertebrates with diverse sex-determining mechanisms, it seems likely that further examples in which mutations that affect *Dmrt1* are associated with SDM transition await discovery.

DMRT1 and the mammalian gonad

Because mice do not require *Dmrt1* for primary sex determination, they may not be typical vertebrates, but the genetic and molecular tools that are available in the mouse have allowed a detailed dissection of *Dmrt1* function^{59–61}. As in other vertebrates, *Dmrt1* expression in mammals is limited to the gonad and is male-specific from the onset of fetal testis differentiation to adulthood. Conditional gene targeting has revealed that *Dmrt1* coordinates

varied aspects of testicular development and has distinct functions in different cell types at different developmental stages. The following sections briefly highlight the different functions of *Dmrt1* in the mouse gonad and consider their mechanistic basis and generality.

The fetal gonad: meiotic initiation and germline fate restriction

Dmrt1 is transiently expressed in the fetal ovary and regulates germ cell development in both sexes during fetal development, but it has different functions in the two sexes. Sex is determined in mouse fetal germ cells shortly after somatic sex determination; after sex determination, female germ cells enter meiotic prophase, where they remain until puberty, whereas male germ cells undergo mitotic arrest until birth. *Dmrt1* is required for the normal execution of both of these processes.

In the fetal ovary meiotic initiation occurs under the influence of retinoic acid and its downstream meiosis inducer stimulated by retinoic acid 8 (*Stra8*)⁶². In the fetal ovary, *Dmrt1* mutant germ cells have severely reduced *Stra8* expression and undergo abnormal meiotic prophase⁶³. mRNA profiling and chromatin immunoprecipitation (ChIP) suggest that transcriptional activation of *Stra8* is the primary function of DMRT1 in the fetal ovary. In *Dmrt1* mutant females, localization of meiotic proteins is abnormal during meiotic prophase, but these females can produce about half of the normal number of oocytes and are fertile.

Dmrt1 mutant males have no obvious phenotype until after birth, except on the 129Sv inbred genetic background, where *Dmrt1* mutants develop testicular teratomas at a very high frequency during fetal development⁶⁴. These tumours arise from cells overexpressing pluripotency proteins and represent a profound failure to maintain germline cell fate commitment. DMRT1 has been linked to several different classes of human testicular germ cell tumour (BOX 3), suggesting that the connection between pluripotency regulation and germ cell cancer may extend beyond mice.

The postnatal testis: spermatogenesis and maintenance of male somatic fate

Dmrt1 has at least two separate requirements in male germ cells after birth. First, it is required for germ cells to resume development just after birth: *Dmrt1* mutant cells do not reinitiate mitosis, nor do they migrate to the basal lamina or undergo spermatogonial differentiation^{44,59}. A second function was revealed when *Dmrt1* was deleted in postnatal undifferentiated spermatogonia. During adult spermatogenesis, spermatogonial stem cells (SSCs) divide to produce a constant supply of committed progenitor cells (that is, undifferentiated spermatogonia), which undergo a series of amplifying divisions coupled with differentiation, occurring in synchronous waves that sweep the seminiferous tubules⁶⁵. Retinoic acid signalling is required for the transition from undifferentiated to differentiating spermatogonium⁶⁶ and is also thought to trigger meiotic initiation in differentiated spermatogonia by activating transcription of *Stra8* (REFS 67,68). DMRT1 is expressed in both undifferentiated and differentiating spermatogonia, but not in meiotic or postmeiotic germ cells. Deletion of *Dmrt1* in undifferentiated spermatogonia causes them to abandon normal development and differentiation and instead express high levels of *Stra8* and precociously and constitutively enter meiosis⁶⁰. DMRT1 appears to control the mitosis versus meiosis decision both by suppressing retinoic acid signalling and by direct transcriptional repression of *Stra8*. Thus DMRT1 has opposite effects on *Stra8* expression in the two sexes — one example of its complex transcriptional activity (BOX 4). In spermatogonia, DMRT1 also promotes expression of key regulators including spermatogenesis- and oogenesis-specific basic helix–loop–helix 1 (*Sohlh1*)⁶⁹. Thus DMRT1 has a central role in regulating the switch between the mitotic and meiotic programs, such that spermatogonial proliferation and differentiation can be completed before meiotic

initiation. A number of questions remain to be addressed; among the most pressing are whether DMRT1 is required in spermatogonial stem cells (SSCs) and how DMRT1 is suppressed to allow meiotic initiation. Also, it is unclear when during evolution *Dmrt1* acquired control of the mitosis–meiosis switch, because *Stra8* is only present in vertebrates, and *Dmrt1* is not expressed in germ cells in many species, including some vertebrates.

Sertoli cells

In mammals, sex is determined in bipotential precursor cells of the gonad that can form either Sertoli cells or granulosa cells, and this pivotal cell fate decision ultimately determines sex throughout the body. *Dmrt1*-null mutant mice are born male and have apparently normal Sertoli cells⁴⁴. However, starting about 2 weeks after birth, the immature Sertoli cells lose expression of the male-specific SOX9 protein and acquire expression of female-specific forkhead box L2 (FOXL2) and other granulosa cell markers⁶⁰. Even deletion of *Dmrt1* in adult Sertoli cells can induce an apparent direct reprogramming to granulosa-like cells. The mutant testes also formed cells resembling theca cells, which are the other somatic cell type of the ovarian follicle. Androgen activity is reduced and oestradiol levels are elevated in mutants, suggesting a male-to-female tilt in hormone signalling, which is probably due in part to elevated expression of the oestrogenic enzyme cytochrome P450 family 19 subfamily A polypeptide 1 (CYP19A1; also known as aromatase). Regulation of oestrogen signalling by *Dmrt1* appears to be a conserved function: in the Nile tilapia, DMRT1 represses *Cyp19a1a* transcription and oestrogen production and promotes male gonadal development⁴⁸.

In the postnatal gonad, DMRT1 transcriptionally represses *Foxl2* as well as some of the genes involved in female primary sex determination (for example, *Wnt4*, R spondin 1 (*Rspo1*) and the oestrogen receptors *Esr1* and *Esr2*). Regulation of these and other genes that are involved in fetal sex determination suggests that the postnatal sex maintenance gene network may use some of the same components as the fetal primary sex determination network (BOX 5). However, the two networks are clearly not identical: loss of *Sox9* only causes sex reversal if it occurs before testicular differentiation^{70,71}, and neither *Dmrt1* nor *Foxl2* is essential for primary sex determination.

Both sexes must maintain gonadal sex. Postnatal loss of *Foxl2* causes a reciprocal reprogramming of granulosa cells to Sertoli-like cells, activating *Sox9* and *Dmrt1*, inducing formation of cells resembling Leydig cells and remodelling the ovary to a more testis-like morphology⁷². This opposite reprogramming suggests that the sexual fate of the somatic gonad is postnatally controlled by the opposed activity of *Dmrt1* and *Foxl2*. *Foxl2* maintains female sex at least in part by transcriptional repression of *Sox9* together with *Esr1* and *Esr2*. How *Foxl2* prevents expression of *Dmrt1* in granulosa cells is unknown, but cell transfection experiments in primary Sertoli cells suggest that FOXL2 might directly repress *Dmrt1* transcription⁷³.

Open questions

Postnatal sex maintenance is a new phenomenon that has barely been explored, and many pressing questions wait to be addressed. One question is whether the phenomenon exists outside mammals. Recent work in medaka suggests this may be the case: XY fish that are mutant for *Dmrt1* develop gonads that initially appear to be male (presumably owing to *Dmy*) but go on to become fertile XY females⁷⁴. Further work will be needed to establish the mechanism of this apparent sex change, its relevance to natural sex change in fish, and its similarity to the process in *Dmrt1* mutant mice. Plasticity of gonadal sex also exists in *C. elegans*, although it may not involve DM domain genes: ectopic expression of *egl-5* in XX hermaphrodites can induce male gene expression in the somatic gonad long after gonadal

sex determination⁷⁵. In mice, genetic studies are needed to understand which of the fetal sex-regulatory genes are postnatally redeployed for sex maintenance and to find genes that are specifically involved in sex maintenance. The basic mechanism also needs to be studied to learn, for example, to what extent transdifferentiation involves resetting transcription factor network activity, whether the transdifferentiation process involves a transient reversion to a less differentiated state and to what extent the epigenetic landscapes of these cells are remodelled to allow the fate change. Granulosa cell proliferation is highly sensitive to retinoic acid. Given that both *Dmrt1* and *Foxl2* are implicated in modulating retinoic acid signalling^{60,76}, it will be important to find out whether elevated retinoic acid signalling plays a part in male-to-female reprogramming in *Dmrt1* mutants. Intriguingly, retinoic acid signalling can act with nuclear receptor subfamily 5 group A member 2 (NR5A2; also known as LRH1) to enhance formation of induced pluripotent stem cells (iPSCs)⁷⁷. LRH1 is normally expressed in the ovary and not in the testis, but it is highly expressed in *Dmrt1* mutant Sertoli cells; this raises the possibility that the reprogramming may involve a pluripotent intermediate state. The timing of transdifferentiation in *Dmrt1* mutants remains puzzling, as it does not become apparent until nearly two weeks after birth. Perhaps other factors maintain sex at earlier stages, or perhaps feminizing activity is not present before this time. *Sox9* and *Sox8* are candidates for helping to maintain male fates, as loss of both genes after sex determination causes testicular defects that are similar to those seen in *Dmrt1* mutants⁷¹.

Conclusions

DM domain genes are clearly central players in sexual differentiation across a broad range of invertebrates and are deeply involved in vertebrate sex determination and its evolution. The available information suggests that DM domain genes arose early in metazoan evolution, probably to regulate some aspect of reproduction or sexual differentiation and have been conserved in this role for hundreds of millions of years. DM domain genes act in the gonad to promote male somatic gonad development in all animals examined so far (except, apparently, in nematodes). The details vary: in vertebrates and some invertebrates, such as *D. magna*, DM domain genes seem to act directly in cells of the somatic gonadal primordium to control their differentiation and/or sexual fate, whereas *dsx* in flies controls the male-specific survival of mesodermal cells that are recruited to join the gonadal primordium^{20,21}. Despite these differences, it is likely that the ancestral function of DM domain genes was to determine gonadal sex and that they subsequently expanded to control sexual dimorphism directly in other tissues. Few species use DM domain genes in the germline, so this function is probably a recent acquisition or an ancient function that has been lost from multiple lineages. Based on studies in non-mammalian vertebrates, we speculate that *Dmrt1* had an ancestral role in sex determination as well as gonadal differentiation in this clade and that the sex-determining role was recently supplanted by *Sry* and *Sox9* in the placental mammals.

Why have DM domain genes persisted as sexual regulators over such large evolutionary distances when other genes (with the possible exception of *Sox9*) seemingly have not? So far, most DM domain sexual regulators seem to function at the interface between sex determination and sexual differentiation, integrating sex with developmental patterning and regulating multiple target genes in varied tissues and cell types, although occasionally, as in medaka, *X. laevis* and probably birds, a DM domain gene rises to the top of the sex determination hierarchy. The functional pleiotropy of these genes may have constrained their evolution and also predisposed them to duplication and functional divergence. However, this situation doubtless applied to other metazoan sex regulators as well, so it remains a mystery why the DM domain genes seem to have survived and prospered in sex regulation to such a remarkable degree. Regardless, work on DM domain genes continues to

reveal molecular mechanisms involved in SDM transitions and the establishment and evolution of sexually dimorphic traits and to uncover new aspects of cell fate regulation.

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About the authors

Clinton K. Matson received his Ph.D. in molecular, cellular and developmental biology and genetics from the University of Minnesota, Minneapolis, USA, where he studied the role of Dmrt genes in mouse development in the laboratory of David Zarkower. His current research focuses on the mechanisms of prostate cancer development in the laboratory of Peter Nelson at the Fred Hutchinson Cancer Research Center, Seattle, Washington, USA.

David Zarkower is Professor of Genetics, Cell Biology and Development and Director of the Developmental Biology Center at the University of Minnesota. He received his doctorate in molecular biology from the University of Wisconsin–Madison, Wisconsin, USA, studying mRNA processing with Marvin Wickens, and conducted postdoctoral research in nematode sex determination with Jonathan Hodgkin at the Medical Research Council (MRC) Laboratory of Molecular Biology in Cambridge, UK. His laboratory studies the molecular genetics and evolution of sex determination and sexual differentiation in nematodes and vertebrates.

Glossary

Spicules	A pair of sensory and copulatory structures in the male tail that are used to help anchor the male to the hermaphrodite vulva during mating.
Oenocytes	Clustered secretory cells found under the abdominal epidermis in insects.
Dysgenetic gonads	Gonads that have developed abnormally but have not undergone a full sexual transformation.
Seminomas	Malignant testicular germ cell tumours of uniform cell composition.
Nonseminomas	Malignant testicular germ cell tumours composed of mixed cell types
Gynandromorphs	Organisms that contain a mixture of male and female cells. Gynandromorphs can be bilateral (male on one side and female on the other) or mosaic (a random mixture of male and female cells).
Leydig cells	Secretory cells that produce testosterone and are found adjacent to the seminiferous tubules of the testis.
Induced pluripotent stem cells	(iPSCs). Pluripotent stem cells that are artificially derived from non-pluripotent cells, typically by genetic manipulation.

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Box 1**An intimate but not exclusive link to sex**

The DM domain is an intertwined zinc-containing DNA-binding module⁷⁸. In *Drosophila melanogaster* and other insects, *doublesex* (*dsx*) is alternatively spliced to yield male- and female-specific proteins that share the DM domain but that have alternative carboxy-terminal sequences^{15,79,80}. The *Caenorhabditis elegans* male sexual regulator *male abnormal 3* (*mab-3*) was found to contain a pair of similar domains, and the shared protein motif was named for these two founding family members⁹. Many other DM domain genes have subsequently been found in a variety of animals, including a growing number involved in sex determination, sexual dimorphism or other aspects of sexual reproduction.

So far, DM domain genes have been found to regulate sexual development in arthropods, nematodes, chordates and *Planaria* spp., to direct gonadal development in all animals except nematodes and to control other sexually dimorphic features autonomously in a wide variety of species. Although DM domains occur widely in metazoans, including in basal groups such as Cnidaria²⁹ and Placozoa, they appear to be absent from other kingdoms; thus, this functional motif probably arose close to the emergence of the metazoans. Most animal species have multiple DM domain genes: for example, flies have four, mice and humans have seven, and nematodes have eleven. Although the full suite of DM domain genes has not yet been systematically studied in any single species, it is clear that they are often associated with sexual development. In *C. elegans*, for example, four of the eleven DM domain genes (namely, *mab-3*, *mab-23* and *dmd-3*) have been studied and all of them regulate sexual development. In mice, mutants for four of the seven DM domain genes (*Dmrt1*, *Dmrt2*, *Dmrt4* and *Dmrt7*) have been described^{44,81–84}. *Dmrt1*, *Dmrt4* and *Dmrt7* have roles in sexual development or reproduction (see below). However, *Dmrt2* apparently instead functions in myogenesis and somitogenesis^{81,85–87}.

In some species, DM domain genes may not be involved in sexual differentiation: in the tunicate *Ciona intestinalis*, a basal chordate, the *Dmrt4* and *Dmrt5* homologue *dmrt1* is required for development of the anterior neural plate⁸⁸, but neither *dmrt1* nor its sole *C. intestinalis* paralogue *dmrt2* have been implicated in sexual regulation.

In summary, DM domain genes are broadly involved in functions related to reproduction and may have a special predilection for sex, but they also regulate other aspects of development. Studies of DM domain genes in basal metazoans, such as the cnidarian *Hydra*, may be particularly informative about their possible ancestral roles in metazoans.

Box 2**Origins and evolution of sexual dimorphism**

Comparative studies in the *Drosophila* genus have helped to show how sexually dimorphic gene expression can evolve³ and have also revealed two discrete mechanisms by which evolutionary changes in doublesex (DSX)-dependent transcriptional output can mediate the diversification of sexually dimorphic features over short evolutionary timescales (see the figure). The first mechanism involves modification of DSX binding sites in *cis*-regulatory elements, affecting either the presence of these sites or their arrangement relative to the binding sites of other regulators and resulting in gain or loss of DSX transcriptional regulation of downstream targets. Two examples are shown. The first concerns the production of long-chain hydrocarbon pheromones (**a**). This depends on expression of the *female-specific desaturase* (*desat^F*) enzyme, which is specific to adult female abdominal oenocytes in many *Drosophila* species. Expression of *desat^F* is rapidly evolving, and the gain or loss of female-specific pheromone production is associated with gain or loss of a DSX^F binding site in the *desat^F* regulatory region⁸⁹. In the example shown, *desat^F* expression was originally controlled by a sex-nonspecific regional transcription factor (indicated by the purple oval), and acquisition of a DSX binding site allowed the regulatory region to come under sex-specific control. Similarly, in the second example (**b**), involving the evolution of abdominal pigmentation, changes in the number, arrangement and spacing of abdominal B (ABDB; indicated by the black oval) and DSX binding sites altered the size the female-specific *bric a brac* (*bab*) expression domain in the abdominal cuticle, and hence the extent of male-specific pigmentation, which is inhibited by *bab*³⁵.

The second evolutionary mechanism involves changes to the expression domain of *dsx* itself and is made possible by the complex and non-ubiquitous pattern of *dsx* expression. An example is evolution of the male-specific *Drosophila* sex combs (modified rows of sensory bristles on the foreleg; panel **c**). The evolutionary acquisition of sex combs has involved the gain of DSX expression in the T1 section of the male foreleg where the sex combs will form and results from the mutual activation of *dsx* and the homeobox (Hox) gene *sex combs reduced* (*scr*) in this region⁹⁰.

Box 3**DMRT1 and the human gonad**

DMRT1 is linked to disorders of sex development (DSD) and to testicular germ cell tumours (TGCTs) in humans^{9,43,91,92}. Distal 9p hemizyosity that removes *DMRT1* is associated with XY sex reversal: the reported gonadal phenotypes range from dysgenetic gonads and ovotestes to small but male gonads. The presumption has been that the feminization associated with loss of *DMRT1* could reflect a failure of male primary sex determination or, perhaps more likely, a failure of male gonadal differentiation and a consequent deficit in male gonadal sex hormones. However, the recently discovered sex maintenance function of *Dmrt1* in mice suggests a third possibility: *DMRT1* may not affect the initial specification of testicular cell fates but may, in some cases, lead to later reprogramming of pre-Sertoli or Sertoli cells into granulosa-like cells. It will be helpful to determine whether 9p deletions cause expression of FOXL2 and other ovarian markers. Paradoxically, whereas XY human 9p deletion patients missing only one copy of *DMRT1* are sometimes female, XY *Dmrt1*-null mutant mice are born male. This difference may indicate that other genes in the 9p interval are involved in human sex determination, or it might reflect an important difference in the timing or function of DMRT1 activity between humans and mice.

DMRT1 is involved in several types of human TGCTs, which is consistent with the role of *Dmrt1* as a tumour suppressor in the mouse fetal testis. In human genome-wide association studies, *DMRT1* is linked to seminomas and nonseminomas^{91–93}. Although these germ cell tumours are pathologically distinct from the juvenile teratomas observed in mice, the involvement of DMRT1 in both conditions suggests possible mechanistic links. Thus, for example, it is possible that the reduced glial-cell-line-derived neurotrophic factor (GDNF) signalling and other changes observed in the mouse are relevant to human TGCTs. Amplification of *DMRT1* is associated with spermatocytic seminomas, which are tumours that are believed to initiate in later germ cells, possibly in premeiotic or meiotic spermatocytes⁹⁴. In the mouse, DMRT1 activity promotes both mitosis and spermatogonial differentiation. It is possible, therefore, that human spermatocytic seminomas originate in a less differentiated cell type whose further differentiation and mitotic proliferation are driven by overexpression of DMRT1. DMRT1 has not been linked to human ovarian disorders, but point mutations in *FOXL2* — which probably activate its function — are found in most ovarian granulosa cell tumours⁹⁵. We speculate that loss of *DMRT1* combined with these *FOXL2* point mutations might be associated with the rarer granulosa cell tumours of the testis.

Box 4**Regulation by and of DM domain proteins**

Most proteins of the DM family have a single DM domain and appear to bind DNA as either homo or heterodimers⁹⁶. A few, however, such as male abnormal 3 (MAB-3), have tandem DM domains and are likely to bind DNA as monomers. *In vitro* binding site selection studies and *in vivo* chromatin immunoprecipitation (ChIP) experiments indicate that most DM domain proteins recognize a similar DNA element whose core sequence is a punctuated palindrome of 7 bp^{96,97}. *In vitro* studies using chemically modified DNA substrates suggested that the DM domain recognizes DNA primarily via the minor groove⁷⁸. However, the specifics of its interactions with DNA are unknown. Their similar DNA binding specificities and the ability to heterodimerize on DNA suggests that DM domain proteins may have complex functional interactions. In addition, ChIP studies in the mouse suggest that DMRT1 regulates transcription of most of the other *Dmrt* genes⁹⁷.

The two alternatively spliced isoforms of *Drosophila melanogaster* DSX — DSX^M and DSX^F — seem to have opposite regulatory functions, and DSX^M generally acts as a transcriptional repressor, whereas DSX^F acts as an activator^{35,98}. MAB-3 acts as a repressor of all target genes identified so far^{27,99}. DMRT1 can either repress or activate transcription and can have differential activity on individual target genes in different cell types, at different developmental stages or in the two sexes^{60,63,97}, but how its activity is modulated is unknown. Despite their sometimes complex expression patterns, very little is known of how tissue-specific expression of any DM domain gene is established. In mice, fetal gonad *Dmrt1* transcription in Sertoli cells is regulated by GATA binding protein 4 (GATA4)^{100,101}, and transgenic analysis indicates that distinct proximal promoter regions can mediate expression in germ cells and somatic cells⁷³. In addition, transgenic analysis in medaka has identified a conserved element in the *Dmrt1* 3' untranslated region (UTR) that can confer somatic gonadal expression on transgenes in fish¹⁰². In the postnatal mouse gonad, sex-specific expression of *Dmrt1* relies, at least in part, on *Foxl2*, as loss of FOXL2 causes ectopic expression of *Dmrt1* in somatic cells of the ovary⁷². Almost nothing is known of how germ cell expression of *Dmrt1* is controlled in any species.

Box 5**Mammalian sex determination and sex maintenance**

In mammals, sex is decided in the gonad during fetal development by the presence or absence of the Y-chromosome-linked testis-determining gene sex-determining region of Y chromosome (*Sry*), which activates the related gene SRY box 9 (*SOX9*) and determines whether bipotential precursor cells will form male Sertoli cells or female granulosa cells¹⁰³. This early decision in a single gonadal cell type ultimately specifies male or female sexual differentiation throughout the body via sex-specific production of steroid hormones (namely, androgens or oestrogens) and other signalling factors. Sex determination can be viewed as a power struggle in the fetal gonad between a male regulatory gene network centred on *Sox9* and a female network involving *Wnt4*, R-spondin 1 (*Rspo1*) and their downstream effector β -catenin^{104,105}. *SOX9* expression in Sertoli cells is necessary and sufficient for testis determination¹⁰⁶ and is reinforced by positive feedback via fibroblast growth factor 9 (*Fgf9*)¹⁰⁵ and a pathway involving the prostaglandin D2 receptor *Ptgds*¹⁰⁷. If *SRY* is not present in the supporting cell lineage during a brief crucial period, sustained *SOX9* expression is not established, and the female regulatory network predominates and drives ovarian development. Postnatally, a long time after sex is determined, a distinct regulatory network functions in each sex to maintain sex. In males, this network requires *Dmrt1*, and in females it requires *Foxl2*. As discussed in the main text, loss of either of these genes can cause even fully differentiated Sertoli or granulosa cells to transdifferentiate into the other cell type, triggering extensive reprogramming of the gonad towards that of the opposite sex.

Online summary

- Most animals reproduce sexually and thus require a sex-determining mechanism.
- Sex-determining mechanisms are surprisingly diverse among animal species, and genes at the top of sex-determining pathways rapidly turn over during evolution.
- Genes containing the DM domain DNA-binding motif appear to have an ancient and conserved role in controlling sexual differentiation and sex determination.
- Invertebrate DM domain genes integrate spatial and temporal inputs with sex-determining pathways to coordinate the sexual differentiation of diverse structures.
- Studies of the DM domain gene *doublesex* in *Drosophila melanogaster* are revealing how new sexually dimorphic features evolve.
- In vertebrates, the DM domain gene *Dmrt1* is required for testicular development.
- In several non-mammalian vertebrates (that is, birds, fish and amphibians) *Dmrt1* or a close homologue has acquired control of sex determination during evolution.
- Detailed studies in the mouse reveal that *Dmrt1* has diverse functions in gonadal development, including a role in preventing male-to-female transdifferentiation of testis cells.

Figure 1. DM domain genes in metazoan sexual development

a | Metazoan phylogeny showing phyla in which DM domain genes have been implicated in sexual development, either through functional analysis or sex-biased expression (highlighted in red). **b** | Arthropod phylogeny showing taxa in which a *doublesex* (*dsx*) gene has been shown to be required for sexual differentiation (highlighted in red). Sex-determining mechanisms of species shown to require *dsx* are indicated by the letters placed next to the name of the taxon. Note that many other sex-determining mechanisms are found in most of these taxa but are not known to involve a *dsx*-like gene. **c** | Vertebrate phylogeny showing taxa in which a *Dmrt1* homologue is required for sex determination (highlighted in red). In teleost fish and in amphibians, many other sex-determining mechanisms occur, but the role of *Dmrt1* is unknown in most of these. ESD, environmental sex determination; HD, haplodiploid system in which females are haploid (from unfertilized eggs), and males are diploid (from fertilized eggs); XY, male heterogamety with XY males and XX females; ZW, female heterogamety system with ZZ males and ZW females.

Figure 2. Diverse roles of DMRT1 orthologues in vertebrate sex determination

Blue shading indicates masculinizing function and pink indicates feminizing function. **a** | In mammals, expression of the X-chromosome-linked sex-determining region of chromosome Y (*SRY*) gene in the fetal gonad triggers male development by activating *SOX9* transcription during a crucial period and, in the absence of *SOX9* activation, ovarian development ensues. *DMRT1* is expressed in the fetal gonad in mammals before sex determination but is required only after gonadal sex is determined. **b** | In medaka, a Y-chromosome-linked, recently duplicated *Dmrt1* gene, DM domain on Y (*Dmy*; also known as *Dmrt1bY*), has a role that is analogous to that of *SRY*, triggering testis development in XY fish. **c** | In birds, *Dmrt1* is Z-chromosome-linked and thus genetic males have two copies, whereas females have one. *Dmrt1* expression is much higher in the genital ridge of ZZ birds, and *Dmrt1* is required for testis determination, suggesting that testis fate requires a critical threshold of *Dmrt1* activity. **d** | In the frog *Xenopus laevis*, a duplicated and truncated *Dmrt1* gene (*dmw*) is found on the female-specific W chromosome and appears to block the ability of the autosomal *Dmrt1* gene to determine testis fate. In medaka, birds and *X. laevis*, the autosomal *Dmrt1* genes also are believed to function in testicular differentiation after gonadal sex is determined, similar to the situation for their mammalian counterparts.