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DMRT1: an ancient sexual regulator required for human gonadogenesis

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Abstract

Transcriptional regulators related to the invertebrate sexual regulators *doublesex* and *mab-3* occur throughout metazoans and control sex in most animal groups. Seven of these *DMRT* genes are found in mammals, and mouse genetics has shown that one, *Dmrt1*, plays a crucial role in testis differentiation, both in germ cells and somatic cells. Deletions and, more recently, point mutations affecting human *DMRT1* have demonstrated that its heterozygosity is associated with 46,XY complete gonadal dysgenesis. Most of our detailed knowledge of DMRT1 function in the testis, the focus of this review, derives from mouse studies, which have revealed that DMRT1 is essential for male somatic and germ cell differentiation and maintenance of male somatic cell fate after differentiation. Moreover, ectopic DMRT1 can reprogram differentiated female granulosa cells into male Sertoli-like cells. The ability of DMRT1 to control sexual cell fate likely derives from at least three properties. First, DMRT1 functionally collaborates with another key male sex regulator, SOX9, and possibly other proteins to maintain and reprogram sexual cell fate. Second, and related, DMRT1 appears to function as a pioneer transcription factor, binding "closed" inaccessible chromatin and promoting its opening to allow binding by other regulators including SOX9. Third, DMRT1 binds DNA by a highly unusual form of interaction and can bind with different stoichiometries.

Keywords

DMRT1; DM domain; testis; DSD

Introduction

Prologue: a brief overview of *DMRT* genes

The *DMRT* gene family was initially discovered in the late 1990s based on sequence similarity between products of the fruitfly sex-determining gene *dsx* and the nematode male regulator *mab-3* [Baker and Ridge, 1980; Shen and Hodgkin, 1988; Raymond et al., 1998].

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

These invertebrate sexual regulators encode transcription factors sharing a motif consisting of an intertwined zinc-binding module followed by an alpha-helical recognition domain that confers sequence-specific DNA binding [Erdman and Burtis, 1993; Erdman et al., 1996; Yi and Zarkower, 1999; Zhu et al., 2000; Murphy et al., 2007; Murphy et al., 2015] (Figure 1a,b). This bipartite motif was named the DM domain after *dsx* and *mab-3*, and the gene family containing the motif were christened *DMRT* genes, for "Doublesex- and mab-3-related transcription factors." Since *dsx* and *mab-3* perform some analogous functions in sexual development and *dsx* can partially substitute for *mab-3* in vivo, it appeared likely that the sequence similarity indeed represented descent from a common ancestral sexual regulator [Raymond et al., 1998]. Subsequent molecular cloning and nucleotide sequence searches have revealed that nearly all metazoan animals have *DMRT* genes, including some from basal clades [Chong et al., 2013; Wexler et al., 2014]. However, *DMRT* genes are not found outside metazoans, suggesting that they arose close to the origin of multicellularity, and thus potentially played an ancient role in specifying differential cell fates, sexual or otherwise.

Most animals have multiple *DMRT* genes, ranging from four in insects and seven in mammals [Raymond et al., 1998; Raymond et al., 1999b; Ottolenghi et al., 2002; Kim et al., 2003; Volff et al., 2003] to eleven in ctenophores such as the starlet sea anemone Nematostella, which diverged early in metazoan evolution [Wexler et al., 2014]. In addition, a number of genes with some similarity to *DMRT* genes but lacking a functional DNA binding domain have been identified, particularly in mammals. Some of these *DMRT*-like genes show sex-specific expression [Veith et al., 2006] but no functional analysis of these genes has been reported. DMRT genes can play varied roles in different tissues but in species where *DMRT* gene function has been investigated most have at least one *DMRT* gene involved in sexual development, acting at the level of sex determination, sex differentiation, or, in some cases, both [Matson and Zarkower, 2012]. The functions of *DMRT* genes can be limited to one tissue or cell type or can be more general. For example, in mice *Dmrt6* and *Dmrt7* (also called *Dmrtb1* and *Dmrtc2, respectively*) regulate germ cell differentiation but appear not to be required in any other cell types [Kim et al., 2007b; Zhang et al., 2014]. At the other end of the spectrum, *dsx* controls most sexually dimorphic aspects of development in insects, regulating a wide variety of distinct functions in different cell types [Baker and Ridge, 1980; Robinett et al., 2010]. Moreover, *dsx* is alternatively spliced to generate sex-specific Dsx protein isoforms that direct male- or female-specific differentiation throughout the body [Burtis and Baker, 1989; Robinett et al., 2010]. This distributed and diverse regulatory repertoire allows *dsx* to act as a primary sex-determining gene in a system that, unlike sex development in vertebrates, does not involve gonadal sex hormones. Because *DMRT* genes regulate sexual differentiation in species as divergent as nematodes [Shen and Hodgkin, 1988; Mason et al., 2008], flatworms [Chong et al., 2013], insects [Geuverink and Beukeboom, 2014], crustaceans [Kato et al., 2011], and vertebrates [Raymond et al., 2000; Matsuda et al., 2002; Smith et al., 2009; Ge et al., 2017; Webster et al., 2017], it seems likely that that sexual regulation is an ancestral function of this gene family [Matson and Zarkower, 2012]. As such, *DMRT* genes are by far the most deeply conserved regulators of sexual development found to date and it seems likely that they have characteristic functional properties that have helped them maintain a crucial role

in this process for hundreds of millions of years in organisms with diverse strategies and mechanisms of sexual development.

DMRT genes in mammals and *DMRT1* as a conserved sexual regulator

Mammals have seven *DMRT* genes [Volff et al., 2003], as well as up to three X-linked *DMRT*-like genes encoding proteins that appear to lack a functional DM domain [Veith et al., 2006]. Mouse gene targeting has probed the functions of all seven *bona fide* autosomal *Dmrt* genes. Mutations in four genes, *Dmrt1, Dmrt4* (*Dmrta1*), *Dmrt6* (*Dmrtb1*) and *Dmrt7* (*Dmrtc2*), disrupt gonadal development in one or both sexes [Raymond et al., 2000; Balciuniene et al., 2006; Kawamata and Nishimori, 2006; Kim et al., 2007b; Zhang et al., 2014], confirming roles in sexual differentiation, while loss of *Dmrt2* disrupts embryonic patterning [Seo et al., 2006]. Mutations in *Dmrt3* and *Dmrt5* (*Dmrta2*) mainly affect the nervous system, disrupting patterning and development of the hippocampus and cerebral cortex [Saulnier et al.; Konno et al., 2012; De Clercq et al., 2018]. *Dmrt3* also is important for spinal interneuron function in locomotion in mice and variants of *DMRT3* determine gait patterns in horses [Andersson et al., 2012; Vallstedt and Kullander, 2013].

Dmrt1 is the only *DMRT* gene shown to play a conserved role in mammalian sexual differentiation. *Dmrt1* is expressed specifically in the gonads of mice and humans, in both somatic and germline cells. In mice, *Dmrt1* mRNA is first detected in the genital ridges around E10.5 in both somatic cells and PGCs of both sexes and it becomes highly male-enriched by about E14.5 as testis differentiation initiates [Raymond et al., 1999a]. Similarly, DMRT1 protein is expressed from E10.5 to E11.5 in somatic and germ cells of both sexes. It becomes male-specific in somatic cells from E12.5 to E13.5, and expression continues in Sertoli cells thereafter [Lei et al., 2007]. DMRT1 is expressed in germ cells of both sexes through E13.5, disappearing by about E15.5 [Lei et al., 2007]. In males DMRT1 is re-expressed in spermatogonia just before birth and maintained in spermatogonia subsequently [Lei et al., 2007; Matson et al., 2010]. During steady state adult spermatogenesis, DMRT1 is expressed in mitotic cells, from *Id4-gfp*-expressing spermatogonial stem cells (SSCs) through differentiated B spermatogonia, disappearing in preleptotene spermatocytes that are entering meiosis [Matson et al., 2010].

Human DMRT1 expression is less fully characterized but appears similar to that of mice. *DMRT1* mRNA was detected only in the testis, among fifty tissues surveyed by RNA blotting [Raymond et al., 1998]. During fetal development *DMRT1* mRNA is detectable by gestational week 11 (GW11) in both sexes. DMRT1 protein is most abundant in pre-Sertoli cells between GW10 and GW20, before becoming strong in spermatogonia from about GW24 onward [Jorgensen et al., 2012]. DMRT1 also is expressed in oogonia and oocytes prior to GW20 and downregulated upon meiotic entry, as in mice [Jorgensen et al., 2012]. In prepubertal testes, DMRT1 protein is highly expressed in spermatogonia and in pre-Sertoli cells, followed by lower expression in Sertoli cells [Jorgensen et al., 2012]. In adult testes, DMRT1 is expressed both in Sertoli cells and in spermatogonia, except in OCT2-positive A-dark cells, which are thought to represent reserve SSCs [Jorgensen et al., 2012]. Similarly, single cell RNA sequencing (scRNA-seq) has found that *Dmrt1* mRNA is expressed postnatally in Sertoli cells and in SSCs, differentiating spermatogonia, and

preleptotene spermatocytes, but not in meiotic and post-meiotic germ cells [Guo et al., 2018; Wang et al., 2018].

Ancient involvement of DMRT1 in vertebrate sex determination and its evolution

Not only is *DMRT1* conserved in mammalian sex regulation but it also is a universal or near-universal sex regulator in other vertebrates. Studies in non-mammalian vertebrates have uncovered *DMRT1* or a close paralog controlling gonadal sex determination in species with diverse sex determination mechanisms, ranging from fish to birds to reptiles and amphibians [Matson and Zarkower, 2012; Ge et al., 2017]. Moreover, in several vertebrate clades changes in *DMRT1* appear to be associated with the acquisition of new sex determination mechanisms. In the fish Medaka, for example, sex is dominantly determined by a *DMRT1* paralog called *Dmy* or *Dmrt1bY* that is located in the non-recombining region of the Y chromosome and acts analogously to *SRY* in mammals [Matsuda et al., 2002; Nanda et al., 2002]. *Dmy* and the X/Y sex chromosome pair containing it are found only in a handful of Medaka species, suggesting a comparatively recent origin for this gene variant and these sex chromosomes [Matsuda et al., 2003; Kondo et al., 2004]. A plausible model is that *Dmy* formed by a duplication of *Dmrt1* that caused constitutive expression of the new gene, rendering individuals carrying it male, regardless of their genotype otherwise, and thus created a neo-Y chromosome. In birds, sex is determined by a ZZ/ZW chromosome system, with ZZ eggs developing as males and ZW eggs as females. Avian *DMRT1* is located on Z and missing from W [Nanda et al., 1999]. Knockdown and overexpression experiments in chickens support the model that *DMRT1* is required in two copies for male gonadal fate [Smith et al., 2009; Lambeth et al., 2014]. Here, a likely model is that deletion of *DMRT1* in an avian progenitor was associated with formation of a new sex chromosome pair. In the African clawed frog *Xenopus laevis*, a *DMRT1* paralog, *DM-W*, is found on the W chromosome of a different ZW sex chromosome system [Yoshimoto et al., 2008]. In ZW individuals, DM-W dominantly interferes with function of the autosomal *DMRT1* gene, preventing testis differentiation [Yoshimoto et al., 2008]. *DM-W* has an N-terminal DM domain but lacks some of the C-terminal sequences found in *DMRT1*, suggesting that it formed via a partial duplication of *DMRT1*, resulting in a neo-W chromosome [Bewick et al., 2011]. In all of these cases it is impossible to distinguish whether a mutation affecting *DMRT1* triggered the formation of new genetic sex-determining mechanisms or instead co-occurred with those events, but it does appear that *DMRT1* and its close paralogs determine sex in a wide range of vertebrates and have done so for hundreds of millions of years. Function of *DMRT1* in vertebrate sex determination is not limited to genetic mechanisms: in the red-eared slider turtle *DMRT1* is required for temperature-dependent sex determination, possibly via temperature-dependent methylation of the *DMRT1* promoter [Ge et al., 2017]. The involvement of *DMRT1* in such a breadth of sex determination systems suggests that it plays a central role in the process and has properties that make it hard to displace.

DMRT1 and DSD: distal 9p deletions define a critical region

The discovery of human DMRT1 and its mapping to 9p24.3 [Raymond et al., 1998] was of immediate relevance for DSD genetics. The association between monosomy of the distal short arm of chromosome 9 (9p), where *DMRT1* is located, and partial or complete 46,XY gonadal dysgenesis has been clear for several decades [Jotterand and Juillard, 1976; Fryns

et al., 1986; Crocker et al., 1988; Hoo et al., 1988; Huret et al., 1988; Bennett et al., 1993; Ogata et al., 1997; Veitia et al., 1997; Veitia et al., 1998]. Distal 9p monosomy also can be associated with cognitive deficits, developmental delay, and a characteristic array of dysmorphic craniofacial features [Alfi et al., 1976]. An early question was whether these effects are genetically linked, much as heterozygosity of *SOX9* causes both campomelic dysplasia and DSD [Foster et al., 1994; Wagner et al., 1994]. Identification of shorter 9p deletions narrowed the critical region for DSD to an interval on 9p24.3, near the telomere, again containing *DMRT1* [McDonald et al., 1997; Flejter et al., 1998; Guioli et al., 1998; Veitia et al., 1998; Calvari et al., 2000; Ottolenghi and McElreavey, 2000]. Because these shorter deletions are found in patients with 46,XY gonadal dysgenesis but lacking the non-urogenital features of monosomy 9p, it is clear that the gonadal and extragonadal aspects of 9p deletion syndrome are genetically separable.

9p deletions consistently affect *DMRT1* but most also remove other genes, including the adjacent paralog *DMRT3*, which is immediately proximal to *DMRT1* on 9p [Ottolenghi et al., 2000]. The co-involvement of *DMRT3* in so many DSD-associated 9p deletions raised the possibility that gonadal dysgenesis might result from combined hemizygosity of *DMRT1* with this gene. This possibility cannot be definitively excluded, but appears unlikely on several grounds. First, although both genes are expressed in the fetal gonad, targeted deletion of *Dmrt3* (and the nearby *Dmrt2*) in the mouse has not revealed a function in gonadal development [Seo et al., 2006; De Clercq et al., 2018; Desmaris et al., 2018]. Moreover, deletion of the interval containing *Dmrt1* and *Dmrt3* in mice, either using CRISPR/Cas9 [Inui et al., 2017] or by meiosis-mediated recombination between *loxP* sites (D.Z., unpublished data) does not cause a more severe phenotype than mutation of *Dmrt1* alone. The most compelling line of evidence is the identification of DSD cases involving 9p microdeletions and point mutations that affect only DMRT1. At least three hemizygous microdeletions have been reported that likely affect just *DMRT1* (Table 1). One, a deletion of 103 kb in a patient with 46,XY complete gonadal dysgenesis, removed exons 1 and 2 of DMRT1 [Ledig et al., 2010], while a second deletion of 35 kb, associated with 46,XY ovotesticular DSD, removed exons 3 and 4 of DMRT1 [Ledig et al., 2012]. A third deletion was found in two 46,XY sisters, one with complete gonadal dysgenesis and female external genitalia and the other with partially virilized DSD: ovotestes and virilized female external genitalia [Calvari et al., 2000]. This deletion removed an interval of 700 kb located distal to *DMRT1* but extending within about 30 kb of the *DMRT1* transcriptional start site, and thus may have disrupted *DMRT1* upstream regulatory elements. The difference in DSD severity between these two sisters helps address the long-standing question of why 9p deletion-associated DSD is variable. The degree of DSD can range from male genitalia with small dysgenic testes to complete gonadal DSD with sex reversal and does not correlate in an obvious way with the extent of the 9p deletion. In addition, there has been no indication of loss of DMRT1 heterozygosity in 9p-associated DSD. Given the range of severity and the finding that siblings with the same deletion can have distinct DSD phenotypes, it is more likely that variable penetrance of distal 9p deletions reflects interaction between *DMRT1* hemizygosity and genetic background, rather than indicating a contiguous gene syndrome or a difference in functional *DMRT1* copy number.

While distal 9p clearly is required in two copies for normal male gonadogenesis, it is less clear whether it plays a role in human ovarian development or function. On the one hand, primary hypogonadism and premature ovarian failure have been reported in 46,XX individuals with terminal 9p deletions [Muroya et al., 2000; Ogata et al., 2001; Fujimoto et al., 2004; Bartels et al., 2013], suggesting a significant role for this region in female gonadal development and function. On the other hand, the maternal inheritance of 9p deletions [Calvari et al., 2000; Quinonez et al., 2013], as well as reports of 46,XX fetuses and adults with distal 9p deletions but apparently normal ovaries [Vialard et al., 2002; Fujimoto et al., 2004; Ounap et al., 2004] suggest that distal 9p hemizygosity does not preclude ovarian differentiation and oogenesis. In sum, it appears that one or more dose-sensitive loci on distal 9p, potentially including *DMRT1*, play a significant but not essential role in ovarian differentiation.

DMRT1 point mutations confirm its role in male gonadogenesis

Despite DNA sequencing efforts by a number of laboratories, *DMRT1* point mutations associated with DSD or male infertility that convincingly cause loss of gene function have been surprisingly rare [Raymond et al., 1999b; Calvari et al., 2000; Ottolenghi et al., 2000; Tewes et al., 2014; Eggers et al., 2016]. Nonetheless, exome sequencing has so far detected four heterozygous point mutations in 46,XY individuals with complete gonadal dysgenesis that are likely to reduce DMRT1 function (Table 1). All four mutations affect amino acids in or adjacent to the DM domain (Figure 1b). One de novo mutation, R111G [Murphy et al., 2015], alters a highly conserved amino acid that makes specific hydrogen-bond contacts with bases in the DNA binding consensus site and also coordinates G118 to help stabilize the DM domain tertiary structure. Substitution with alanine at this position nearly eliminates in vitro DNA binding, confirming the importance of this residue [Murphy et al., 2015]. Substitution of R111 to G severely reduces DNA binding affinity of the mutant protein but also allows it to alter the stoichiometry of binding by wild-type protein in vitro, suggesting that R111G may function as a dominant negative mutation [Murphy et al., 2015]. A second mutation, Y84C, alters a position in the zinc-binding module of the DM domain [Fan et al., 2017]. Mutations at this position have not been observed in model organisms or tested in vitro, but the introduction of a cysteine is likely to disrupt folding of the zinc-binding module and consequently affect DNA affinity. The Y84C mutation was maternally inherited, further indicating that DMRT1 heterozygosity can be compatible with female fertility. The third mutation, a de novo R80S change, alters a conserved residue in the DM domain that makes hydrogen-bond contacts with the DNA backbone [Murphy et al., 2015; Buonocore et al., 2019]. Molecular dynamics simulation predicted that this change should reduce DNA affinity [Buonocore et al., 2019]. To directly test that prediction we have compared in vitro DNA binding affinity of full-length human wild type, R80S, and R111G substituted DMRT1 proteins to labeled synthetic DNA oligonucleotides by electrophoretic mobility shift analysis (EMSA; Figure 2). The R80S mutant protein bound less efficiently than wild-type. As discussed below, DMRT1 can bind DNA as a dimer, trimer or tetramer. R80S mutant DMRT1 binding was reduced less severely than that of R111G DMRT1 on DNAs that bind DMRT1 trimers and dimers but more severely on a site that binds DMRT1 tetramers. Both mutant proteins showed severely reduced binding on a DMRT1 tetramer site from the *Foxl2* locus. Collectively the data were consistent with the R80S mutation being responsible for

DSD in the affected patient. The fourth mutation, L139Q, is a de novo change affecting a conserved residue adjacent to the DM domain [Chauhan et al., 2017]. This residue is not included in the published X-ray structure, it has not been altered in model organism genetic screens, and its role in DNA binding has not yet been tested; thus, how the L139Q mutation affects DMRT1 function remains to be established. In addition, a heterozygous P74L mutation has been found in two brothers with Sertoli-only syndrome. It is predicted to affect minor groove recognition and reduces DMRT1 DNA binding affinity, suggesting that reduced DMRT1 function also can cause infertility without DSD (T. Maric and M. Murphy, in preparation).

A handful of other *DMRT1* mutations or rare variants outside the DM domain have been reported in DSD [Raymond et al., 1999b] and male infertility cases [Tewes et al., 2014]. It is possible that these variants represent loss-of-function mutations, but given the lower sequence conservation of the affected regions and the lack of a simple and robust functional assay for DMRT1, it cannot yet be excluded that these are merely uncommon neutral variants. The low discovery rate for *DMRT1* mutations in DSD and the absence of homozygous *DMRT1* mutations is notable. One suggested explanation is that *DMRT1* deletions also are associated with male infertility, allowing the load of *DMRT1* mutations to be purged by spermatogenic failure [Lopes et al., 2013].

Insights into DMRT1 from mouse conditional genetics: DMRT1 and spermatogenesis

Conditional gene targeting technology in mice has allowed a detailed dissection of *Dmrt1* function in a mammal with similar reproductive biology to that of humans. Conditional genetics has confirmed the importance of *Dmrt1* in gonadal development, has found similarities and differences to its function in humans, and has uncovered some unexpected roles for DMRT1. In the mouse, DMRT1 plays a major role in male but not female gonadal development, as appears to be the case in humans [Raymond et al., 2000]. However, in mice the *Dmrt1* gene is fully recessive rather than haploinsufficient. This difference in dose-sensitivity is not unusual for genes involved in mouse and human sex regulation. For example, *SOX9, WT1 and SF1* are haploinsufficient for human male gonadogenesis but recessive for testis differentiation in the mouse [Parker et al., 1999; Barrionuevo et al., 2006]. Homozygous deletion of *Dmrt1* in XX mice causes a deficit in primordial follicles but does not significantly reduce female fertility [Krentz et al., 2011]. By contrast, XY *Dmrt1* homozygous mutants have severely dysgenic gonads [Raymond et al., 2000]. However, XY homozygous *Dmrt1* mutant mice still undergo male nongonadal development, in contrast to 46,XY humans with heterozygous *DMRT1* point mutations or deletions, who can have complete gonadal dysgenesis at an early enough stage to cause complete male-to-female phenotypic sex reversal. Indeed, testicular development is superficially normal in *Dmrt1*$^{-/-}$ mice of mixed genetic background until birth, with gonadal dysgenesis becoming apparent mainly between one and four weeks postnatally [Raymond et al., 2000; Fahrioglu et al., 2007; Kim et al., 2007a]. It therefore appears that there are fundamental differences, at least in timing, between the requirements for *DMRT1* in human and *Dmrt1* in mouse. While these differences must be kept in mind, the mouse has nonetheless been useful for deeper exploration of DMRT1 function than is possible in humans.

Conditional gene targeting has allowed the dissection of requirements for *Dmrt1* in germ cells and Sertoli cells and has revealed distinct functions in different cell types and at different stages of development. The role of *Dmrt1* in the germ line has been reviewed in detail elsewhere [Zarkower, 2013; Zhang and Zarkower, 2017] and will be summarized briefly here. Consistent with its expression in fetal PGCs and postnatal spermatogonia, *Dmrt1* is required both for the establishment of postnatal spermatogenesis and subsequently for the maintenance of the progenitor pool that supplies adult steady state spermatogenesis [Fahrioglu et al., 2007; Kim et al., 2007a]. Fetal loss of *Dmrt1* blocks perinatal mitotic reactivation, migration and viability of prospermatogonia [Kim et al., 2007a]. Later, in steady-state adult spermatogenesis, *Dmrt1* has multiple distinct roles affecting spermatogonial stem cells: it activates the stem cell regulator *Plzf* to help maintain the SSC pool and it is required also in *Ngn3*-positive committed progenitor cells to let them replenish the SSC pool when they are artificially depleted [Zhang et al., 2016]. In steady state spermatogenesis *Dmrt1* also has a key role in differentiating spermatogonia. In these cells, rather than promoting self-renewal, *Dmrt1* promotes differentiation and proliferation by activating genes including *Sohlh1*, and inhibits premature meiotic initiation by inhibiting retinoic acid signaling and repressing *Stra8* [Matson et al., 2010]. Normally meiotic initiation is triggered in part by degradation of DMRT1 via a beta-TrCP ubiquitin ligase complex [Nakagawa et al., 2017]. The importance of this regulation is revealed when beta-TrCP is depleted, which stabilizes DMRT1 in preleptotene cells, reduces STRA8 expression in these cells, and prevents meiosis. The suite of germline DMRT1 functions collectively allows spermatogonia to proliferate and differentiate, supported by a stable stem cell pool, and promotes long-term fertility. How DMRT1 activity is modified to perform different roles at different stages of spermatogonial development is important but just beginning to be understood, as discussed below.

Insights from mouse conditional genetics: DMRT1 and cancer

DMRT1 not only controls normal spermatogenesis but also is linked to germ cell cancer in both mice and humans. In mice of mixed or C57BL/6J genetic background the *Dmrt1* mutant testis appears normal until shortly after birth, but in male mice of the 129Sv/J inbred genetic background, mutant fetal germ cells fail to undergo cell cycle arrest and downregulation of pluripotency regulators, and this results in a very high incidence of testicular teratomas [Krentz et al., 2009]. These tumors differ in a number of respects from the testicular germ cell tumors most common in humans [Oosterhuis and Looijenga, 2005]. However, GWAS studies have shown strong association of *DMRT1* with human testicular germ cell cancer [Turnbull et al., 2010; Kanetsky et al., 2011; Kratz et al., 2011]. Gain of distal 9p, involving *DMRT1*, also is found in all cases of the rare germ cell cancer spermatocytic seminoma, which is found exclusively in older men [Looijenga et al., 2006]. These tumors are thought to arise from a different stage of germ cell development and may result from ectopic *DMRT1* activity [Oosterhuis and Looijenga, 2005].

Insights from mouse conditional genetics: DMRT1 and somatic sexual cell fate

The role of DMRT1 in Sertoli cells is most germane to DSD. *Dmrt1* null mutants or conditional mutants with *Dmrt1* deleted in fetal bipotential progenitor cells have apparently normal testes at birth, but mutant Sertoli cells fail to complete differentiation and are

unable to support spermatogenesis beyond meiotic prophase [Raymond et al., 2000; Kim et al., 2007a]. Unexpectedly, however, deletion of *Dmrt1* in pre-Sertoli or Sertoli cells not only prevents the completion of differentiation but also causes their transdifferentiation to granulosa-like cells, the female supporting cell counterpart [Matson et al., 2011]. This sexual transdifferentiation occurs even when *Dmrt1* is deleted in fully differentiated post-mitotic adult Sertoli cells, demonstrating that male sexual cell fate must be continuously maintained from at least puberty onward. Loss of *Dmrt1* in Sertoli cells causes ectopic expression of key female sex regulators including *Foxl2, Wnt4, Rspo1, Esr1* and *Esr2*, and genetic analysis indicates that these genes drive the male-to-female transdifferentiation that ensues [Minkina et al., 2014]. Whether the transdifferentiated granulosa-like cells are able to support oogenesis is unknown, because germ cells in the mutant XY gonad undergo apoptosis and efforts to co-transplant mutant XY somatic cells together with wild type XX oocytes have not been successful (D.Z., unpublished).

Why do Sertoli cells need a sex maintenance system? When *Dmrt1* is deleted, the feminizing genes are activated by inappropriate retinoic acid (RA) signaling. Blocking RA signaling or deleting the RA receptor *Rara* suppresses this activation and largely blocks transdifferentiation [Minkina et al., 2014]. A likely model is that DMRT1 not only limits RA signaling in spermatogonia but also restricts the activity of RA in somatic cells, thereby allowing RA to promote meiosis in spermatocytes without causing premature meiotic initiation in spermatogonia or sexual cell fate transdifferentiation in Sertoli cells.

Granulosa cells also need sex maintenance: deletion of *Foxl2* in the ovary causes a reciprocal, female-to-male, transdifferentiation. These results suggest that DMRT1 and FOXL2 anchor antagonistic regulatory networks that ensure the stability of somatic sex throughout postnatal life [Uhlenhaut et al., 2009]. Consistent with this view, deletion of *Foxl2* suppresses transdifferentiation of XY *Dmrt1* mutants [Minkina et al., 2014] and the transcriptome of XX gonads expressing ectopic *Dmrt1* is very similar to that of XX gonads lacking *Foxl2* [Lindeman et al., 2015]. Whether *DMRT1* is required in the human gonad for the testis determination decision or subsequently for testis differentiation, and whether it maintains male sexual fate in the human gonad are unknown. This uncertainty is in part because early failure of human testicular development, for example in *SRY* or *SOX9* mutants, results in highly dysgenic “streak” gonads and female internal and external genitalia, a condition known as Swyer Syndrome (MIM 400044).

DMRT1 not only is required to maintain male fate in the XY gonad but also can impose male fate on somatic cells of the XX gonad. Ectopic expression of DMRT1 in the ovary from a single copy transgene, starting either around E11.5 in the fetal gonad, driven by *Sf1-Cre*, or in the adult gonad, driven by *UBC-CreERT2* or *Hsd17b1-Cre*, can reprogram granulosa cells into Sertoli-like cells organized into tubule-like structures [Lindeman et al., 2015] and can sexually transform the ovarian transcriptome (Lindeman and Zarkower, unpublished). These results indicate that DMRT1 can act instructively to determine male cell fate. However, transdifferentiation did not start until about one to two weeks after birth, similar to the timing of transdifferentiation in XY *Dmrt1* mutants. Even expressing DMRT1 from about E9.5 using *Wt1-Cre* did not cause fetal masculinization. This is perhaps unsurprising, given that DMRT1 normally is expressed at similar levels in bipotential

Sertoli/granulosa progenitor cells of the two sexes prior to sex determination. One study has found that a multicopy *Dmrt1* cDNA driven by the *Wt1* promoter can cause partial female-to-male sex reversal [Zhao et al., 2015]. However this observation involved progeny of a single transgenic founder with extremely high expression of *Dmrt1* and some of its targets, in contrast to the approximately physiological DMRT1 expression level in the previous study. Also, a different multicopy transgenic study also expressing *Dmrt1* from the *Wt1* promoter found no effect on sex determination [Agbor et al., 2013]. Possible explanations for the discrepancy are that highly overexpressed DMRT1 and/or its targets competes for regulatory cofactors shared with transcription factors involved in female sex determination, or that the *Wt1-Dmrt1* transgene in the single affected founder line was inserted in or near a locus involved in female gonadogenesis.

The DM domain and gene regulation

DMRT proteins are site-specific DNA binding proteins and genetic experiments have confirmed that they regulate transcription of target genes by activation or repression. The DMRT1 DNA binding consensus sequence in vitro and in vivo is a 13 bp pseudopalindromic DNA motif, which is also bound by other DMRT proteins [Murphy et al., 2007]. DMRT proteins can form heteromers on DNA both in vitro and in vivo, raising the possibility that they may interact functionally by forming mixed complexes with different activities [Murphy et al., 2007; Zhang et al., 2014; Zhang and Zarkower, 2017]. X-ray crystallography has revealed that the DM domain inserts its recognition helix into the DNA major groove and makes sequence-specific hydrogen bond contacts with key DNA bases in the manner of many other site-specific DNA binding proteins (Figure 1c,d) [Murphy et al., 2015]. The DM domain can bind DNA as a trimer, with an antiparallel dimer of protomers on one side of the binding site and a single protomer on the other side. Unexpectedly, however, the antiparallel dimerized protomers jointly insert their recognition helices into the same segment of an inherently wide major groove. This binding mode, involving two helices contacting the same major groove segment, may be unique to the DM domain, as it has not been observed among the hundreds of other protein/DNA complexes structurally analyzed to date. Both in vitro and in vivo DMRT1 is able to bind DNA not only as a trimer but also as a dimer or tetramer, based on EMSA (see Figure 2) and high resolution ChIP-exo analysis [Murphy et al., 2015]. The stoichiometry of binding depends both on the specific sequence and the inherent shape of the particular binding site.

Understanding how DMRT1 functions requires an understanding of how it can impose a new fate on cells even after differentiation is complete and how it can perform very different functions in different contexts, for example in somatic versus germ cells or in SSCs versus differentiating spermatogonia. There are several properties that could help account for these abilities. These properties include the differences in stoichiometry of DNA binding, interactions with cell type-specific transcriptional regulators, the ability to bind closed chromatin, effects on genome organization, or a combination of these. The significance of the alternative DNA binding stoichiometries remains unclear, as individual stoichiometries do not correspond in an obvious way with targets that are, for example, activated or repressed by DMRT1. R111 plays a key role in DNA base recognition (Figure 1d) and the dominant change in DNA binding stoichiometry caused by the R111G mutation

hints that context-dependent binding modes may be important. The other DSD-associated DMRT1 mutations have not yet been tested for such dominance.

There is stronger evidence that some of the other properties are significant. Genetic and molecular analysis suggest that DMRT1 functionally interacts with SOX9 both in the fetal and adult testis. Deletion of *Sox9* causes more severe sexual transdifferentiation of *Dmrt1* mutant testes [Minkina et al., 2014] and deletion of *Sox9* with its close paralog *Sox8* also limits reprogramming of granulosa cells by DMRT1 in the ovary [Lindeman et al., 2015; Lindeman et al., 2021]. ChIP-seq experiments show that DMRT1 and SOX9 often bind near one another on target genes in the fetal testis [Rahmoun et al., 2017], in juvenile Sertoli cells, and when expressed ectopically in the ovary [Lindeman et al., 2021], suggesting that they have joint effects on target gene expression. DMRT1 also functionally interacts with DMRT6 in spermatogonia, but here the relationship may be antagonistic [Zhang et al., 2014]. DMRT1 promotes spermatogonial differentiation and activates spermatogonial regulators such as *Sohlh1*, whereas DMRT6 is expressed late in spermatogonial development, in Intermediate and B spermatogonia, and helps repress the mitotic and differentiation programs prior to meiosis by turning off spermatogonial regulators like *Sohlh1* and *Sohlh2*, as well as *Dmrt1*. DMRT1 and DMRT6 bind to an overlapping set of genomic sites, and ChIP-reChIP shows that they can bind the same site at the same time on several key target genes, suggesting that they form heteromers in which the presence of DMRT6 may switch regulation from activation to repression.

DMRT1 appears to act as a pioneer transcription factor, binding “closed” low-modification chromatin and opening it to binding by other transcriptional regulators [Lindeman et al., 2021]. In granulosa cells, ectopic DMRT1 can bind differentially accessible regions (DARs) that normally are open in Sertoli cells but closed in granulosa cells. Many of these sites are not efficiently bound by SOX9 when it is expressed alone, but expression of DMRT1 with SOX9 allows binding by SOX9, consistent with DMRT1 pioneering for SOX9 and presumably other regulatory factors [Lindeman et al., 2021]. Pioneer function requires the ability to bind nucleosomal DNA and, while this has not yet been demonstrated for DMRT1 in vitro, structural modeling suggests that DMRT1 should be able to interact with its site in a nucleosomal context. Finally, DNA binding by DMRT1 as a multimer suggests the possibility that it could mediate chromatin contacts that could help form functional domains. Preliminary HiC data indicate that ectopic DMRT1 does result in formation of new chromatin loops (M.W.M and D.Z., unpublished), but further work will be required to determine their significance for gene expression and how directly DMRT1 participates in the formation of these structures. A model for how DMRT1 controls maintenance and reprogramming of sexual cell fate is shown in Figure 3.

Conclusion

Epilogue: Open questions and future directions

The past two decades have revealed much about *DMRT* genes in general and *DMRT1* in particular. We now have convincing evidence that *DMRT1* is an important regulator of testicular differentiation in most or all vertebrates, is involved in the evolution of new vertebrate sex determination mechanisms, has a number of distinct functions in the

mouse gonad both during and after development, and is a key locus involved in 9p deletion-associated DSD. We also are gaining a more detailed view of how DMRT1 engages with target genes, know the identities of many of these genes, and are beginning to identify other regulators that cooperate with DMRT1 to control gonadal gene expression.

Much remains to be learned, however. Among the open questions relating to the role of DMRT1 in DSD are whether DMRT1 controls sex determination or sex differentiation in the human gonad (or both), how genetic background intersects with *DMRT1* loss in DSD, whether male sex maintenance exists in the human testis, and whether other genes on distal 9p contribute to testicular differentiation or male fertility. Most of these are questions not easily resolved using mice or other model organisms, but advances in organoid technology may soon accelerate progress [Sakib et al., 2019].

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References:

Agbor VA, Tao S, Lei N, Heckert LL: A Wt1-Dmrt1 transgene restores DMRT1 to sertoli cells of Dmrt1(−/−) testes: a novel model of DMRT1-deficient germ cells. Biol Reprod 88:51 (2013). [PubMed: 23255335]

Alfi OS, Donnell GN, Allderdice PW, Derencsenyi A: The 9p- syndrome. Ann Genet 19:11–16 (1976). [PubMed: 1084115]

Andersson LS, Larhammar M, Memic F, Wootz H, Schwochow D, Rubin CJ, Patra K, Arnason T, Wellbring L, Hjalm G, Imsland F, Petersen JL, McCue ME, Mickelson JR, Cothran G, Ahituv N, Roepstorff L, Mikko S, Vallstedt A, Lindgren G, Andersson L, Kullander K: Mutations in DMRT3 affect locomotion in horses and spinal circuit function in mice. Nature 488:642–646 (2012). [PubMed: 22932389]

Baker BS, Ridge KA: Sex and the single cell. I. On the action of major loci affecting sex determination in Drosophila melanogaster. Genetics 94:383–423 (1980). [PubMed: 6771185]

Balciuniene J, Bardwell VJ, Zarkower D: Mice mutant in the DM domain gene Dmrt4 are viable and fertile but have polyovular follicles. Mol Cell Biol 26:8984–8991 (2006). [PubMed: 16982677]

Barrionuevo F, Bagheri-Fam S, Klattig J, Kist R, Taketo MM, Englert C, Scherer G: Homozygous inactivation of Sox9 causes complete XY sex reversal in mice. Biol Reprod 74:195–201 (2006). [PubMed: 16207837]

Bartels I, Putz I, Reintjes N, Netzer C, Shoukier M: Normal intelligence and premature ovarian failure in an adult female with a 7.6 Mb de novo terminal deletion of chromosome 9p. Eur J Med Genet 56:458–462 (2013). [PubMed: 23811035]

Bennett CP, Docherty Z, Robb SA, Ramani P, Hawkins JR, Grant D: Deletion 9p and sex reversal. J Med Genet 30:518–520 (1993). [PubMed: 8326498]

Bewick AJ, Anderson DW, Evans BJ: Evolution of the closely related, sex-related genes DM-W and DMRT1 in African clawed frogs (Xenopus). Evolution 65:698–712 (2011). [PubMed: 21044062]

Buonocore F, Clifford-Mobley O, King TFJ, Striglioni N, Man E, Suntharalingham JP, Del Valle I, Lin L, Lagos CF, Rumsby G, Conway GS, Achermann JC: Next-Generation Sequencing Reveals

Novel Genetic Variants (SRY, DMRT1, NR5A1, DHH, DHX37) in Adults With 46,XY DSD. J Endocr Soc 3:2341–2360 (2019). [PubMed: 31745530]

Burtis KC, Baker BS: Drosophila doublesex gene controls somatic sexual differentiation by producing alternatively spliced mRNAs encoding related sex-specific polypeptides. Cell 56:997–1010 (1989). [PubMed: 2493994]

Calvari V, Bertini V, De Grandi A, Peverali G, Zuffardi O, Ferguson-Smith M, Knudtzon J, Camerino G, Borsani G, Guioli S: A new submicroscopic deletion that refines the 9p region for sex reversal. Genomics 65:203–212 (2000). [PubMed: 10857744]

Chauhan V, Jyotsna VP, Jain V, Khadgawat R, Dada R: Novel Heterozygous Genetic Variants in Patients with 46,XY Gonadal Dysgenesis. Horm Metab Res 49:36–42 (2017). [PubMed: 27711951]

Chong T, Collins JJ 3rd, Brubacher JL, Zarkower D, Newmark PA: A sex-specific transcription factor controls male identity in a simultaneous hermaphrodite. Nat Commun 4:1814 (2013). [PubMed: 23652002]

Crocker M, Coghill SB, Cortinho R: An unbalanced autosomal translocation (7;9) associated with feminization. Clin Genet 34:70–73 (1988). [PubMed: 3409542]

De Clercq S, Keruzore M, Desmaris E, Pollart C, Assimacopoulos S, Preillon J, Ascenzo S, Matson CK, Lee M, Nan X, Li M, Nakagawa Y, Hochepied T, Zarkower D, Grove EA, Bellefroid EJ: DMRT5 Together with DMRT3 Directly Controls Hippocampus Development and Neocortical Area Map Formation. Cereb Cortex 28:493–509 (2018). [PubMed: 28031177]

Desmaris E, Keruzore M, Saulnier A, Ratie L, Assimacopoulos S, De Clercq S, Nan X, Roychoudhury K, Qin S, Kricha S, Chevalier C, Lingner T, Henningfeld KA, Zarkower D, Mallamaci A, Theil T, Campbell K, Pieler T, Li M, Grove EA, Bellefroid EJ: DMRT5, DMRT3, and EMX2 Cooperatively Repress Gsx2 at the Pallium-Subpallium Boundary to Maintain Cortical Identity in Dorsal Telencephalic Progenitors. J Neurosci 38:9105–9121 (2018). [PubMed: 30143575]

Eggers S, Sadedin S, van den Bergen JA, Robevska G, Ohnesorg T, Hewitt J, Lambeth L, Bouty A, Knarston IM, Tan TY, Cameron F, Werther G, Hutson J, O'Connell M, Grover SR, Heloury Y, Zacharin M, Bergman P, Kimber C, Brown J, Webb N, Hunter MF, Srinivasan S, Titmuss A, Verge CF, Mowat D, Smith G, Smith J, Ewans L, Shalhoub C, Crock P, Cowell C, Leong GM, Ono M, Lafferty AR, Huynh T, Visser U, Choong CS, McKenzie F, Pachter N, Thompson EM, Couper J, Baxendale A, Gecz J, Wheeler BJ, Jefferies C, MacKenzie K, Hofman P, Carter P, King RI, Krausz C, van Ravenswaaij-Arts CM, Looijenga L, Drop S, Riedl S, Cools M, Dawson A, Juniarto AZ, Khadilkar V, Khadilkar A, Bhatia V, Dung VC, Atta I, Raza J, Thi Diem Chi N, Hao TK, Harley V, Koopman P, Warne G, Faradz S, Oshlack A, Ayers KL, Sinclair AH: Disorders of sex development: insights from targeted gene sequencing of a large international patient cohort. Genome Biol 17:243 (2016). [PubMed: 27899157]

Erdman SE, Burtis KC: The Drosophila doublesex proteins share a novel zinc finger related DNA binding domain. EMBO J 12:527–535 (1993). [PubMed: 8440242]

Erdman SE, Chen HJ, Burtis KC: Functional and genetic characterization of the oligomerization and DNA binding properties of the Drosophila doublesex proteins. Genetics 144:1639–1652 (1996). [PubMed: 8978051]

Fahrioglu U, Murphy MW, Zarkower D, Bardwell VJ: mRNA expression analysis and the molecular basis of neonatal testis defects in Dmrt1 mutant mice. Sex Dev 1:42–58 (2007). [PubMed: 18391515]

Fan Y, Zhang X, Wang L, Wang R, Huang Z, Sun Y, Yao R, Huang X, Ye J, Han L, Qiu W, Zhang H, Liang L, Gu X, Yu Y: Diagnostic Application of Targeted Next-Generation Sequencing of 80 Genes Associated with Disorders of Sexual Development. Sci Rep 7:44536 (2017). [PubMed: 28295047]

Flejter WL, Fergestad J, Gorski J, Varvill T, Chandrasekharappa S: A gene involved in XY sex reversal is located on chromosome 9, distal to marker D9S1779. Am J Hum Genet 63:794–802 (1998). [PubMed: 9718346]

Foster JW, Dominguez-Steglich MA, Guioli S, Kwok C, Weller PA, Stevanovic M, Weissenbach J, Mansour S, Young ID, Goodfellow PN, et al. : Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 372:525–530 (1994). [PubMed: 7990924]

Fryns JP, Kleczkowska A, Casaer P, van den Berghe H: Double autosomal chromosomal aberration (3p trisomy/9p monosomy) and sex-reversal. Ann Genet 29:49–52 (1986). [PubMed: 3487277]

Fujimoto Y, Okuyama T, Iijima M, Tanaka T, Horikawa R, Yamada K, Ogata T: Genitourinary phenotype in XX patients with distal 9p monosomy. Mol Genet Metab 82:173–179 (2004). [PubMed: 15172006]

Ge C, Ye J, Zhang H, Zhang Y, Sun W, Sang Y, Capel B, Qian G: Dmrt1 induces the male pathway in a turtle species with temperature-dependent sex determination. Development 144:2222–2233 (2017). [PubMed: 28506988]

Geuverink E, Beukeboom LW: Phylogenetic distribution and evolutionary dynamics of the sex determination genes doublesex and transformer in insects. Sex Dev 8:38–49 (2014). [PubMed: 24401160]

Guioli S, Schmitt K, Critcher R, Bouzyk M, Spurr NK, Ogata T, Hoo JJ, Pinsky L, Gimelli G, Pasztor L, Goodfellow PN: Molecular analysis of 9p deletions associated with XY sex reversal: refining the localization of a sex-determining gene to the tip of the chromosome. Am J Hum Genet 63:905–908 (1998). [PubMed: 9718347]

Guo J, Grow EJ, Mlcochova H, Maher GJ, Lindskog C, Nie X, Guo Y, Takei Y, Yun J, Cai L, Kim R, Carrell DT, Goriely A, Hotaling JM, Cairns BR: The adult human testis transcriptional cell atlas. Cell Res 28:1141–1157 (2018). [PubMed: 30315278]

Hoo JJ, Salefsky IS, Lin CC: Possible localisation of a recessive testis forming gene on 9p24. American Journal of Human Genetics (Suppl) 45:A78 (1988).

Huret JL, Leonard C, Forestier B, Rethore MO, Lejeune J: Eleven new cases of del(9p) and features from 80 cases. J Med Genet 25:741–749 (1988). [PubMed: 3070043]

Inui M, Tamano M, Kato T, Takada S: CRISPR/Cas9-mediated simultaneous knockout of Dmrt1 and Dmrt3 does not recapitulate the 46,XY gonadal dysgenesis observed in 9p24.3 deletion patients. Biochem Biophys Rep 9:238–244 (2017). [PubMed: 28956011]

Jorgensen A, Nielsen JE, Blomberg Jensen M, Graem N, Rajpert-De Meyts E: Analysis of meiosis regulators in human gonads: a sexually dimorphic spatio-temporal expression pattern suggests involvement of DMRT1 in meiotic entry. Mol Hum Reprod 18:523–534 (2012). [PubMed: 22899867]

Jotterand M, Juillard E: A new case of trisomy for the distal part of 13q due to maternal translocation, t(9;13)(p21;q21). Hum Genet 33:213–222 (1976). [PubMed: 964983]

Kanetsky PA, Mitra N, Vardhanabhuti S, Vaughn DJ, Li M, Ciosek SL, Letrero R, D'Andrea K, Vaddi M, Doody DR, Weaver J, Chen C, Starr JR, Hakonarson H, Rader DJ, Godwin AK, Reilly MP, Schwartz SM, Nathanson KL: A second independent locus within DMRT1 is associated with testicular germ cell tumor susceptibility. Hum Mol Genet 20:3109–3117 (2011). [PubMed: 21551455]

Kato Y, Kobayashi K, Watanabe H, Iguchi T: Environmental sex determination in the branchiopod crustacean Daphnia magna: deep conservation of a Doublesex gene in the sex-determining pathway. PLoS Genet 7:e1001345 (2011). [PubMed: 21455482]

Kawamata M, Nishimori K: Mice deficient in Dmrt7 show infertility with spermatogenic arrest at pachytene stage. FEBS Lett 580:6442–6446 (2006). [PubMed: 17098235]

Kim S, Bardwell VJ, Zarkower D: Cell type-autonomous and non-autonomous requirements for Dmrt1 in postnatal testis differentiation. Dev Biol 307:314–327 (2007a). [PubMed: 17540358]

Kim S, Kettlewell JR, Anderson RC, Bardwell VJ, Zarkower D: Sexually dimorphic expression of multiple doublesex-related genes in the embryonic mouse gonad. Gene Expr Patterns 3:77–82 (2003). [PubMed: 12609607]

Kim S, Namekawa SH, Niswander LM, Ward JO, Lee JT, Bardwell VJ, Zarkower D: A mammal-specific Doublesex homolog associates with male sex chromatin and is required for male meiosis. PLoS Genet 3:e62 (2007b). [PubMed: 17447844]

Kondo M, Nanda I, Hornung U, Schmid M, Schartl M: Evolutionary origin of the medaka Y chromosome. Curr Biol 14:1664–1669 (2004). [PubMed: 15380069]

Konno D, Iwashita M, Satoh Y, Momiyama A, Abe T, Kiyonari H, Matsuzaki F: The mammalian DM domain transcription factor Dmrta2 is required for early embryonic development of the cerebral cortex. PLoS One 7:e46577 (2012). [PubMed: 23056351]

Kratz CP, Han SS, Rosenberg PS, Berndt SI, Burdett L, Yeager M, Korde LA, Mai PL, Pfeiffer R, Greene MH: Variants in or near KITLG, BAK1, DMRT1, and TERT-CLPTM1L predispose to familial testicular germ cell tumour. J Med Genet 48:473–476 (2011). [PubMed: 21617256]

Krentz AD, Murphy MW, Kim S, Cook MS, Capel B, Zhu R, Matin A, Sarver AL, Parker KL, Griswold MD, Looijenga LH, Bardwell VJ, Zarkower D: The DM domain protein DMRT1 is a dose-sensitive regulator of fetal germ cell proliferation and pluripotency. Proc Natl Acad Sci U S A 106:22323–22328 (2009). [PubMed: 20007774]

Krentz AD, Murphy MW, Sarver AL, Griswold MD, Bardwell VJ, Zarkower D: DMRT1 promotes oogenesis by transcriptional activation of Stra8 in the mammalian fetal ovary. Dev Biol 356:63–70 (2011). [PubMed: 21621532]

Lambeth LS, Raymond CS, Roeszler KN, Kuroiwa A, Nakata T, Zarkower D, Smith CA: Over-expression of DMRT1 induces the male pathway in embryonic chicken gonads. Dev Biol 389:160–172 (2014). [PubMed: 24576538]

Ledig S, Hiort O, Scherer G, Hoffmann M, Wolff G, Morlot S, Kuechler A, Wieacker P: Array-CGH analysis in patients with syndromic and non-syndromic XY gonadal dysgenesis: evaluation of array CGH as diagnostic tool and search for new candidate loci. Hum Reprod 25:2637–2646 (2010). [PubMed: 20685758]

Ledig S, Hiort O, Wunsch L, Wieacker P: Partial deletion of DMRT1 causes 46,XY ovotesticular disorder of sexual development. Eur J Endocrinol 167:119–124 (2012). [PubMed: 22573722]

Lei N, Hornbaker KI, Rice DA, Karpova T, Agbor VA, Heckert LL: Sex-specific differences in mouse DMRT1 expression are both cell type- and stage-dependent during gonad development. Biol Reprod 77:466–475 (2007). [PubMed: 17567962]

Lindeman RE, Gearhart MD, Minkina A, Krentz AD, Bardwell VJ, Zarkower D: Sexual Cell-Fate Reprogramming in the Ovary by DMRT1. Curr Biology 25:764–771 (2015).

Lindeman RE, Murphy MW, Agrimson KS, Gewiss RL, Bardwell VJ, Gearhart MD, Zarkower D: The conserved sex regulator DMRT1 recruits SOX9 in sexual cell fate reprogramming. Nucleic Acids Res 49:6144–6164 (2021). [PubMed: 34096593]

Looijenga LH, Hersmus R, Gillis AJ, Pfundt R, Stoop HJ, van Gurp RJ, Veltman J, Beverloo HB, van Drunen E, van Kessel AG, Pera RR, Schneider DT, Summersgill B, Shipley J, McIntyre A, van der Spek P, Schoenmakers E, Oosterhuis JW: Genomic and expression profiling of human spermatocytic seminomas: primary spermatocyte as tumorigenic precursor and DMRT1 as candidate chromosome 9 gene. Cancer Res 66:290–302 (2006). [PubMed: 16397242]

Lopes AM, Aston KI, Thompson E, Carvalho F, Goncalves J, Huang N, Matthiesen R, Noordam MJ, Quintela I, Ramu A, Seabra C, Wilfert AB, Dai J, Downie JM, Fernandes S, Guo X, Sha J, Amorim A, Barros A, Carracedo A, Hu Z, Hurles ME, Moskovtsev S, Ober C, Paduch DA, Schiffman JD, Schlegel PN, Sousa M, Carrell DT, Conrad DF: Human spermatogenic failure purges deleterious mutation load from the autosomes and both sex chromosomes, including the gene DMRT1. PLoS Genet 9:e1003349 (2013). [PubMed: 23555275]

Mason DA, Rabinowitz JS, Portman DS: dmd-3, a doublesex-related gene regulated by tra-1, governs sex-specific morphogenesis in C. elegans. Development 135:2373–2382 (2008). [PubMed: 18550714]

Matson CK, Murphy MW, Griswold MD, Yoshida S, Bardwell VJ, Zarkower D: The mammalian doublesex homolog DMRT1 is a transcriptional gatekeeper that controls the mitosis versus meiosis decision in male germ cells. Dev Cell 19:612–624 (2010). [PubMed: 20951351]

Matson CK, Murphy MW, Sarver AL, Griswold MD, Bardwell VJ, Zarkower D: DMRT1 prevents female reprogramming in the postnatal mammalian testis. Nature 476:101–104 (2011). [PubMed: 21775990]

Matson CK, Zarkower D: Sex and the singular DM domain: insights into sexual regulation, evolution and plasticity. Nat Rev Genet 13:163–174 (2012). [PubMed: 22310892]

Matsuda M, Nagahama Y, Shinomiya A, Sato T, Matsuda C, Kobayashi T, Morrey CE, Shibata N, Asakawa S, Shimizu N, Hori H, Hamaguchi S, Sakaizumi M: DMY is a Y-specific DM-domain gene required for male development in the medaka fish. Nature 417:559–563 (2002). [PubMed: 12037570]

Matsuda M, Sato T, Toyazaki Y, Nagahama Y, Hamaguchi S, Sakaizumi M: Oryzias curvinotus has DMY, a gene that is required for male development in the medaka, O. latipes. Zoolog Sci 20:159–161 (2003). [PubMed: 12655179]

McDonald MT, Flejter W, Sheldon S, Putzi MJ, Gorski JL: XY sex reversal and gonadal dysgenesis due to 9p24 monosomy. Am J Med Genet 73:321–326 (1997). [PubMed: 9415692]

Minkina A, Matson CK, Lindeman RE, Ghyselinck NB, Bardwell VJ, Zarkower D: DMRT1 protects male gonadal cells from retinoid-dependent sexual transdifferentiation. Dev Cell 29:511–520 (2014). [PubMed: 24856513]

Muroya K, Okuyama T, Goishi K, Ogiso Y, Fukuda S, Kameyama J, Sato H, Suzuki Y, Terasaki H, Gomyo H, Wakui K, Fukushima Y, Ogata T: Sex-determining gene(s) on distal 9p: clinical and molecular studies in six cases. J Clin Endocrinol Metab 85:3094–3100 (2000). [PubMed: 10999792]

Murphy MW, Lee JK, Rojo S, Gearhart MD, Kurahashi K, Banerjee S, Loeuille GA, Bashamboo A, McElreavey K, Zarkower D, Aihara H, Bardwell VJ: An ancient protein-DNA interaction underlying metazoan sex determination. Nat Struct Mol Biol 22:442–451 (2015). [PubMed: 26005864]

Murphy MW, Zarkower D, Bardwell VJ: Vertebrate DM domain proteins bind similar DNA sequences and can heterodimerize on DNA. BMC Mol Biol 8:58 (2007). [PubMed: 17605809]

Nakagawa T, Zhang T, Kushi R, Nakano S, Endo T, Nakagawa M, Yanagihara N, Zarkower D, Nakayama K: Regulation of mitosis-meiosis transition by the ubiquitin ligase beta-TrCP in male germ cells. Development 144:4137–4147 (2017). [PubMed: 28982686]

Nanda I, Kondo M, Hornung U, Asakawa S, Winkler C, Shimizu A, Shan Z, Haaf T, Shimizu N, Shima A, Schmid M, Schartl M: A duplicated copy of DMRT1 in the sex-determining region of the Y chromosome of the medaka, Oryzias latipes. Proc Natl Acad Sci U S A 99:11778–11783 (2002). [PubMed: 12193652]

Nanda I, Shan Z, Schartl M, Burt DW, Koehler M, Nothwang H, Grutzner F, Paton IR, Windsor D, Dunn I, Engel W, Staeheli P, Mizuno S, Haaf T, Schmid M: 300 million years of conserved synteny between chicken Z and human chromosome 9. Nat Genet 21:258–259 (1999). [PubMed: 10080173]

Ogata T, Muroya K, Matsuo N, Hata J, Fukushima Y, Suzuki Y: Impaired male sex development in an infant with molecularly defined partial 9p monosomy: implication for a testis forming gene(s) on 9p. J Med Genet 34:331–334 (1997). [PubMed: 9138160]

Ogata T, Muroya K, Ohashi H, Mochizuki H, Hasegawa T, Kaji M: Female gonadal development in XX patients with distal 9p monosomy. Eur J Endocrinol 145:613–617 (2001). [PubMed: 11720880]

Oosterhuis JW, Looijenga LH: Testicular germ-cell tumours in a broader perspective. Nat Rev Cancer 5:210–222 (2005). [PubMed: 15738984]

Ottolenghi C, Fellous M, Barbieri M, McElreavey K: Novel paralogy relations among human chromosomes support a link between the phylogeny of doublesex-related genes and the evolution of sex determination. Genomics 79:333–343 (2002). [PubMed: 11863363]

Ottolenghi C, McElreavey K: Deletions of 9p and the quest for a conserved mechanism of sex determination. Mol Genet Metab 71:397–404 (2000). [PubMed: 11001833]

Ottolenghi C, Veitia R, Quintana-Murci L, Torchard D, Scapoli L, Souleyreau-Therville N, Beckmann J, Fellous M, McElreavey K: The region on 9p associated with 46,XY sex reversal contains several transcripts expressed in the urogenital system and a novel doublesex-related domain. Genomics 64:170–178 (2000). [PubMed: 10729223]

Ounap K, Uibo O, Zordania R, Kiho L, Ilus T, Oiglane-Shlik E, Bartsch O: Three patients with 9p deletions including DMRT1 and DMRT2: a girl with XY complement, bilateral ovotestes, and extreme growth retardation, and two XX females with normal pubertal development. Am J Med Genet A 130A:415–423 (2004). [PubMed: 15481033]

Parker KL, Schimmer BP, Schedl A: Genes essential for early events in gonadal development. Cell Mol Life Sci 55:831–838 (1999). [PubMed: 10412366]

Quinonez SC, Park JM, Rabah R, Owens KM, Yashar BM, Glover TW, Keegan CE: 9p partial monosomy and disorders of sex development: review and postulation of a pathogenetic mechanism. Am J Med Genet A 161A:1882–1896 (2013). [PubMed: 23824832]

Rahmoun M, Lavery R, Laurent-Chaballier S, Bellora N, Philip GK, Rossitto M, Symon A, Pailhoux E, Cammas F, Chung J, Bagheri-Fam S, Murphy M, Bardwell V, Zarkower D, Boizet-Bonhoure B, Clair P, Harley VR, Poulat F: In mammalian foetal testes, SOX9 regulates expression of its target genes by binding to genomic regions with conserved signatures. Nucleic Acids Res (2017).

Raymond CS, Kettlewell JR, Hirsch B, Bardwell VJ, Zarkower D: Expression of Dmrt1 in the genital ridge of mouse and chicken embryos suggests a role in vertebrate sexual development. Dev Biol 215:208–220 (1999a). [PubMed: 10545231]

Raymond CS, Murphy MW, O'Sullivan MG, Bardwell VJ, Zarkower D: Dmrt1, a gene related to worm and fly sexual regulators, is required for mammalian testis differentiation. Genes Dev 14:2587–2595 (2000). [PubMed: 11040213]

Raymond CS, Parker ED, Kettlewell JR, Brown LG, Page DC, Kusz K, Jaruzelska J, Reinberg Y, Flejter WL, Bardwell VJ, Hirsch B, Zarkower D: A region of human chromosome 9p required for testis development contains two genes related to known sexual regulators. Hum Mol Genet 8:989–996 (1999b). [PubMed: 10332030]

Raymond CS, Shamu CE, Shen MM, Seifert KJ, Hirsch B, Hodgkin J, Zarkower D: Evidence for evolutionary conservation of sex-determining genes. Nature 391:691–695 (1998). [PubMed: 9490411]

Robinett CC, Vaughan AG, Knapp JM, Baker BS: Sex and the single cell. II. There is a time and place for sex. PLoS Biol 8:e1000365 (2010). [PubMed: 20454565]

Sakib S, Voigt A, Goldsmith T, Dobrinski I: Three-dimensional testicular organoids as novel in vitro models of testicular biology and toxicology. Environ Epigenet 5:dvz011 (2019). [PubMed: 31463083]

Saulnier A, Keruzore M, De Clercq S, Bar I, Moers V, Magnani D, Walcher T, Filippis C, Kricha S, Parlier D, Viviani L, Matson CK, Nakagawa Y, Theil T, Gotz M, Mallamaci A, Marine JC, Zarkower D, Bellefroid EJ: The Doublesex Homolog Dmrt5 is Required for the Development of the Caudomedial Cerebral Cortex in Mammals. Cereb Cortex.

Seo KW, Wang Y, Kokubo H, Kettlewell JR, Zarkower DA, Johnson RL: Targeted disruption of the DM domain containing transcription factor Dmrt2 reveals an essential role in somite patterning. Dev Biol 290:200–210 (2006). [PubMed: 16387292]

Shen MM, Hodgkin J: mab-3, a gene required for sex-specific yolk protein expression and a male-specific lineage in C. elegans. Cell 54:1019–1031 (1988). [PubMed: 3046751]

Smith CA, Roeszler KN, Ohnesorg T, Cummins DM, Farlie PG, Doran TJ, Sinclair AH: The avian Z-linked gene DMRT1 is required for male sex determination in the chicken. Nature 461:267–271 (2009). [PubMed: 19710650]

Tewes AC, Ledig S, Tuttelmann F, Kliesch S, Wieacker P: DMRT1 mutations are rarely associated with male infertility. Fertil Steril 102:816–820 e813 (2014). [PubMed: 24934491]

Turnbull C, Rapley EA, Seal S, Pernet D, Renwick A, Hughes D, Ricketts M, Linger R, Nsengimana J, Deloukas P, Huddart RA, Bishop DT, Easton DF, Stratton MR, Rahman N: Variants near DMRT1, TERT and ATF7IP are associated with testicular germ cell cancer. Nat Genet 42:604–607 (2010). [PubMed: 20543847]

Uhlenhaut NH, Jakob S, Anlag K, Eisenberger T, Sekido R, Kress J, Treier AC, Klugmann C, Klasen C, Holter NI, Riethmacher D, Schutz G, Cooney AJ, Lovell-Badge R, Treier M: Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. Cell 139:1130–1142 (2009). [PubMed: 20005806]

Vallstedt A, Kullander K: Dorsally derived spinal interneurons in locomotor circuits. Ann N Y Acad Sci 1279:32–42 (2013). [PubMed: 23531000]

Veith AM, Klattig J, Dettai A, Schmidt C, Englert C, Volff JN: Male-biased expression of X-chromosomal DM domain-less Dmrt8 genes in the mouse. Genomics 88:185–195 (2006). [PubMed: 16488114]

Veitia R, Nunes M, Brauner R, Doco-Fenzy M, Joanny-Flinois O, Jaubert F, Lortat-Jacob S, Fellous M, McElreavey K: Deletions of distal 9p associated with 46,XY male to female sex reversal: definition of the breakpoints at 9p23.3-p24.1. Genomics 41:271–274 (1997). [PubMed: 9143505]

Veitia RA, Nunes M, Quintana-Murci L, Rappaport R, Thibaud E, Jaubert F, Fellous M, McElreavey K, Goncalves J, Silva M, Rodrigues JC, Caspurro M, Boieiro F, Marques R, Lavinha J: Swyer syndrome and 46,XY partial gonadal dysgenesis associated with 9p deletions in the absence of monosomy-9p syndrome. Am J Hum Genet 63:901–905 (1998). [PubMed: 9718353]

Vialard F, Ottolenghi C, Gonzales M, Choiset A, Girard S, Siffroi JP, McElreavey K, Vibert-Guigue C, Sebaoun M, Joye N, Portnoi MF, Jaubert F, Fellous M: Deletion of 9p associated with gonadal dysfunction in 46,XY but not in 46,XX human fetuses. J Med Genet 39:514–518 (2002). [PubMed: 12114486]

Volff JN, Zarkower D, Bardwell VJ, Schartl M: Evolutionary dynamics of the DM domain gene family in metazoans. J Mol Evol 57 Suppl 1:S241–249 (2003). [PubMed: 15008421]

Wagner T, Wirth J, Meyer J, Zabel B, Held M, Zimmer J, Pasantes J, Bricarelli FD, Keutel J, Hustert E, Wolf U, Tommerup N, Schempp W, Scherer G: Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the SRY-related gene SOX9. Cell 79:1111–1120 (1994). [PubMed: 8001137]

Wang M, Liu X, Chang G, Chen Y, An G, Yan L, Gao S, Xu Y, Cui Y, Dong J, Chen Y, Fan X, Hu Y, Song K, Zhu X, Gao Y, Yao Z, Bian S, Hou Y, Lu J, Wang R, Fan Y, Lian Y, Tang W, Wang Y, Liu J, Zhao L, Wang L, Liu Z, Yuan R, Shi Y, Hu B, Ren X, Tang F, Zhao XY, Qiao J: Single-Cell RNA Sequencing Analysis Reveals Sequential Cell Fate Transition during Human Spermatogenesis. Cell Stem Cell 23:599–614 e594 (2018). [PubMed: 30174296]

Webster KA, Schach U, Ordaz A, Steinfeld JS, Draper BW, Siegfried KR: Dmrt1 is necessary for male sexual development in zebrafish. Dev Biol 422:33–46 (2017). [PubMed: 27940159]

Wexler JR, Plachetzki DC, Kopp A: Pan-metazoan phylogeny of the DMRT gene family: a framework for functional studies. Dev Genes Evol 224:175–181 (2014). [PubMed: 24903586]

Yi W, Zarkower D: Similarity of DNA binding and transcriptional regulation by Caenorhabditis elegans MAB-3 and Drosophila melanogaster DSX suggests conservation of sex determining mechanisms. Development 126:873–881 (1999). [PubMed: 9927589]

Yoshimoto S, Okada E, Umemoto H, Tamura K, Uno Y, Nishida-Umehara C, Matsuda Y, Takamatsu N, Shiba T, Ito M: A W-linked DM-domain gene, DM-W, participates in primary ovary development in Xenopus laevis. Proc Natl Acad Sci U S A 105:2469–2474 (2008). [PubMed: 18268317]

Zarkower D: DMRT genes in vertebrate gametogenesis. Curr Top Dev Biol 102:327–356 (2013). [PubMed: 23287039]

Zhang T, Murphy MW, Gearhart MD, Bardwell VJ, Zarkower D: The mammalian Doublesex homolog DMRT6 coordinates the transition between mitotic and meiotic developmental programs during spermatogenesis. Development 141:3662–3671 (2014). [PubMed: 25249458]

Zhang T, Oatley J, Bardwell VJ, Zarkower D: DMRT1 Is Required for Mouse Spermatogonial Stem Cell Maintenance and Replenishment. PLoS Genet 12:e1006293 (2016). [PubMed: 27583450]

Zhang T, Zarkower D: DMRT proteins and coordination of mammalian spermatogenesis. Stem Cell Res 24:195–202 (2017). [PubMed: 28774758]

Zhao L, Svingen T, Ng ET, Koopman P: Female-to-male sex reversal in mice caused by transgenic overexpression of Dmrt1. Development 142:1083–1088 (2015). [PubMed: 25725066]

Zhu L, Wilken J, Phillips NB, Narendra U, Chan G, Stratton SM, Kent SB, Weiss MA: Sexual dimorphism in diverse metazoans is regulated by a novel class of intertwined zinc fingers. Genes Dev 14:1750–1764 (2000). [PubMed: 10898790]

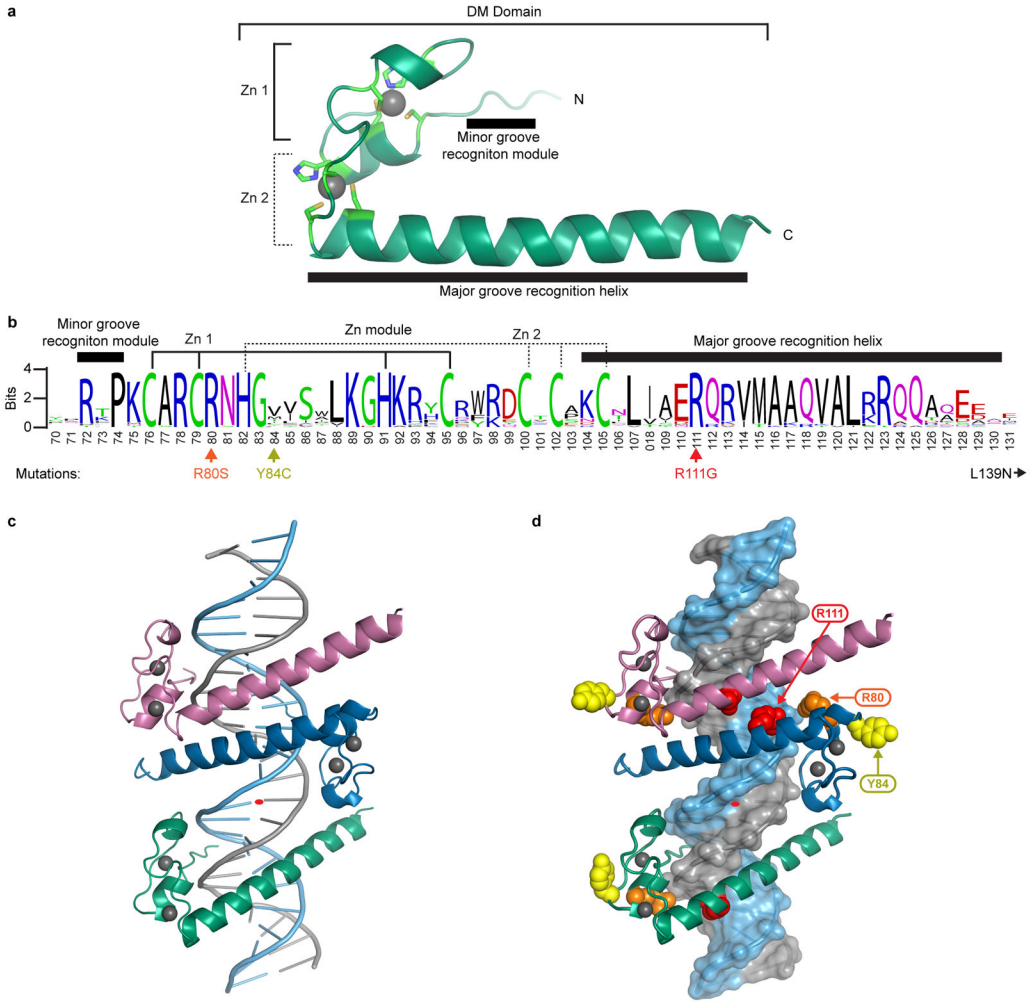

Figure 1: The DM domain and DMRT1/DNA interaction

a. X-ray crystal structure of the human DMRT1 DM domain from Murphy et al. (2015) with major structural domains indicated. **b.** Conservation of metazoan DM domains. Positions of structural domains and sites of human mutations associated with DSD are indicated. **c.** X-ray crystal structure of human DMRT1 DM domains bound to DNA from Murphy et al. (2015), with DMRT1 protomers in distinct colors. Two protomers (pink and blue) insert in DNA major groove on one side of the binding site, while a single protomer (green) inserts in the major groove on the other side. Red oval indicates central basepair of the punctuated pseudopalindromic DNA site. **d.** DM domain amino acids affected by human DMRT1 point mutations associated with DSD. Affected amino acid side-chains are shown as space-filling models and labeled. R80 makes contact with the DNA phosphate backbone, Y84 is in the zinc module, and R111 makes contacts with conserved DNA bases in the recognition site.

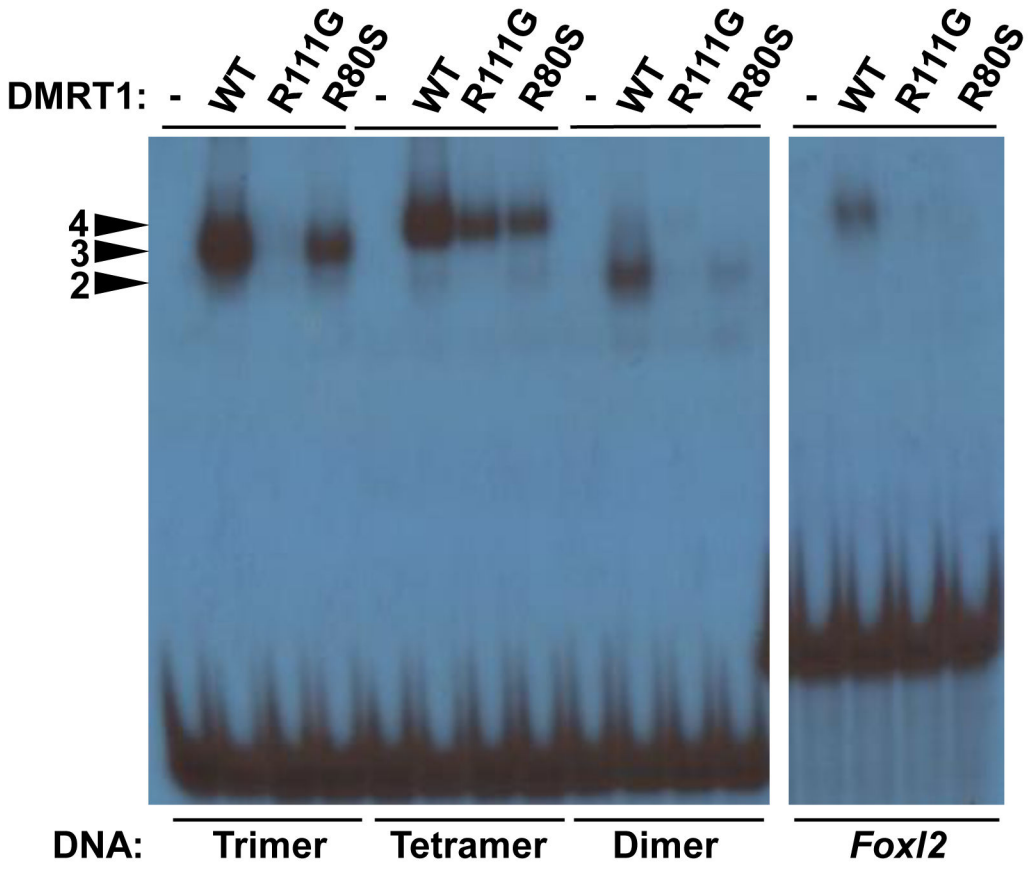

Figure 2: Sex-reversing R111G and R80S mutations reduce DNA binding by DMRT1 in vitro.
Left panel: DMRT1 protein translated in vitro was incubated with radiolabeled double-stranded DNAs previously shown to bind dimers, trimers or tetramers of DMRT1 as previously described [Murphy et al., 2015]. Reactions contained unprogrammed reticulocyte lysate (−), lysate programed with wild-type synthetic human *Dmrt1* mRNA (WT), or synthetic mRNA encoding the R111G or R80S mutant DMRT1 proteins, as indicated. After incubation, reactions were subjected to native gene electrophoresis and autoradiography. Mobility of dimers, trimers and tetramers are indicated by arrowheads. The R111G mutation reduced trimer and dimer binding more severely than tetramer binding, while the R80S mutation reduced dimer and tetramer binding more severely than trimer binding. Right panel: DMRT1 proteins were incubated with DNA containing a DMRT1 binding site from the *Foxl2* locus. Both mutations severely reduced binding to this site.

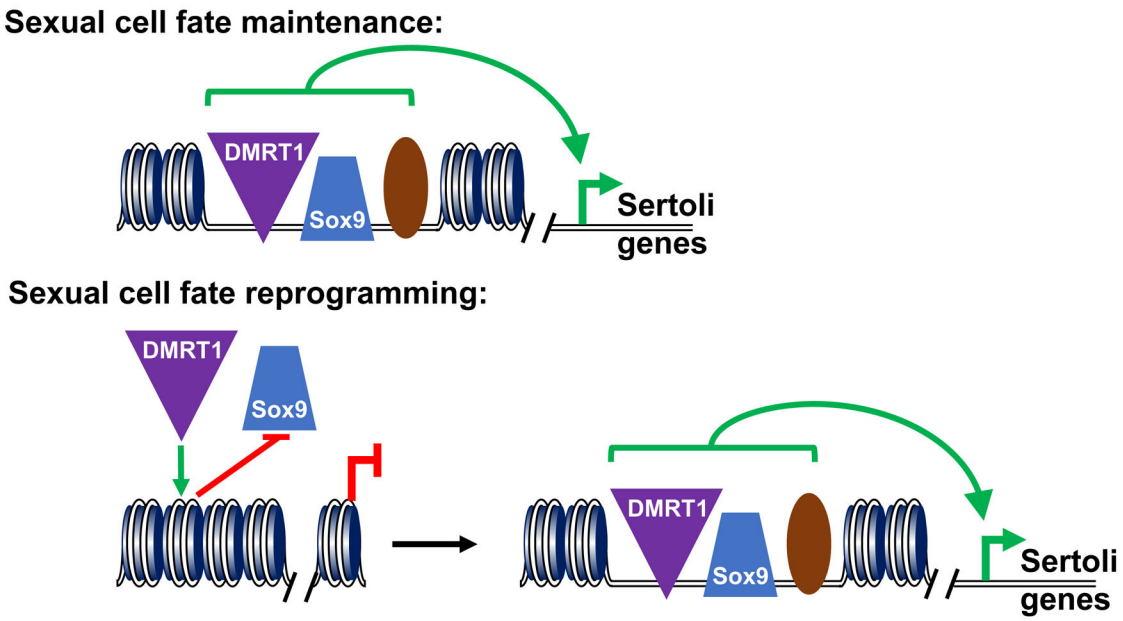

Figure 3: Model of DMRT1 regulation of sexual cell fate maintenance and reprogramming.
Top: In sexual cell fate maintenance, DMRT1 (purple) occupies distal regulatory elements, in many cases with SOX9 (blue) and other regulators (brown). This binding helps keep chromatin accessible ("open") and promotes expression of genes involved in Sertoli cell fate and function. Bottom: In reprogramming, DMRT1 binds inaccessible ("closed") chromatin and promotes its conversion to a form accessible to SOX9 and other regulators. Occupancy by DMRT1 and other regulators relieves repression and promotes activation of genes in involved in Sertoli cell fate and function. How DMRT1 affects chromatin accessibility and potentially chromatin interactions is unknown, as are whether DMRT1 binding stoichiometry affects these functions and whether DMRT1 binding can promote formation of repressive chromatin at granulosa-expressed genes.

Table 1.

DMRT1 microdeletions and missense mutations associated with human DSD.

Lesion	Phenotype	Notes	Citations
103kb deletion removing *DMRT1* exons 1 and 2	46,XY complete DSD	Hemizygous	Ledig et al., 2010
35kb deletion removing *DMRT1* exons 3 and 4	46,XY ovotesticular DSD	Hemizygous	Ledig et al., 2012
700kb deletion extending distally from 30kb 5' of *DMRT1* TSS	46,XY complete DSD; 46,XY partial DSD	Hemizygous; probands are siblings	Calvari et al., 2000
DMRT1 R111G missense mutation in DM domain	46,XY complete DSD	Heterozygous; reduced DNA affinity, altered stoichiometry	Murphy et al., 2015
DMRT1 Y84C missense mutation in DM domain	46,XY complete DSD	Heterozygous; maternally inherited	Fan et al., 2017
DMRT1 R80S missense mutation in DM domain	46,XY complete DSD	Heterozygous; reduced DNA affinity	Buonocore et al., 2019; this paper
DMRT1 L139Q missense mutation adjacent to DM domain	46,XY complete DSD	Heterozygous	Chauhan et al. 2017
DMRT1 P74L missense mutation in DM domain	46,XY Sertoli-only syndrome	Heterozygous; reduced DNA affinity	T. Maric and M. Murphy, in prep.