The Roles of *DMRT1* in the Fate Determination and Maintenance of Male Germ Cells

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Abstract

Spermatogenesis is a highly ordered and evolutionarily conserved biological process, which strictly depends on the precise cell fate determination and long-term maintenance of male germ cells. As one of the most evolutionarily conserved sex-regulatory factors in vertebrates, *DMRT1* plays an irreplaceable core role in male gonad development, germ cell fate determination, and the entire process of spermatogenesis. Existing studies mainly focus on its function in testicular support cells, and there is still a lack of systematic understanding of its specific regulatory mechanisms in male germ cells. This paper systematically elaborates on the molecular characteristics and spatiotemporal expression patterns of *DMRT1*, integrating its key functions in embryonic male germ cell development, spermatogonial stem cell maintenance and renewal, meiosis initiation and progression, and sperm morphogenesis and maturation. It also summarizes the upstream and downstream regulatory networks centered on *DMRT1*. The goal is to clarify the core value of *DMRT1* in the fate determination and maintenance of male germ cells. Furthermore, this paper addresses current research gaps, including unclear epigenetic mechanisms and species differences, and provides a theoretical basis for explaining the pathogenesis of male infertility and identifying potential intervention targets.

Keywords

***DMRT1*; Spermatogonial Cells; Spermatogenesis; Transcriptional Regulation; Cell Fate Determination.**

1. Introduction

Spermatogenesis in mammals plays a decisive role in male fertility and species preservation. As a highly ordered developmental cascade, its core relies on the precise fate determination and lifelong maintenance of male germ cells[1]. From the specialization of primordial germ cells to the self-renewal and differentiation of spermatogonial stem cells, this process is driven by a complex genetic and epigenetic regulatory network. Disruption of this network can lead to infertility or the development of germ cell tumors[2]. Germ cell fate determination is the core scientific issue of this regulatory network, explaining how germ cells undergo directed development while maintaining stem cell properties and genomic integrity. This field witnessed rapid development around 1970, but despite decades of research, the exact mechanisms are still not fully understood.

DMRT1 (Doublesex and Mab-3 Related Transcription Factor 1) is one of the most evolutionarily conserved sex development regulatory factors in both vertebrates and invertebrates. The homologous genes of *DMRT1* are found in species such as Drosophila melanogaster, Caenorhabditis elegans, and mice[3]. *DMRT1* initially attracted attention for its core role in gonadal sex determination and differentiation. It drives male gonad development by suppressing female-specific genetic programs and activating male-specific pathways in cells[4]. Compared to the global regulation at the gonadal tissue level, *DMRT1*'s expression

and functional regulation in male germ cells are directly linked to the directed development and maintenance of germ cells, making it a key factor connecting gonadal sex determination and the specific process of spermatogenesis[5-7].

Current research on *DMRT1* mainly focuses on testicular support cells[8]. However, existing studies lack systematic reviews and integrative analyses of *DMRT1*'s biological functions in male germ cells, particularly in the core regulatory mechanisms involved in spermatogonial stem cell maintenance and differentiation.

This review systematically summarizes the spatiotemporal expression patterns, multiple biological functions, and downstream molecular mechanisms of *DMRT1* in male germ cells, particularly in spermatogonial stem cells, and discusses future research directions in this field.

2. The Role of *DMRT1* at Different Stages of Male Germ Cell Development

2.1. Development of Male Germ Cells during Embryonic Stage

Primordial germ cells originate from the proximal epiblast around E6.5[9] and need to migrate to the gonadal ridge and settle there. *DMRT1* plays a key regulatory role in guiding PGCs migration and colonization towards the male gonad, promoting their masculinization[10]. Naoko and colleagues revealed that *DMRT1* acts synergistically with SOX17 (a key factor in PGC specification) to initiate the germline commitment process. On the one hand, it directly binds to downstream target genes to regulate the temporal expression of stage-specific genes, while on the other hand, it triggers epigenetic reprogramming, laying the foundation for PGCs to acquire gametogenesis potential[11]. As a result, *DMRT1* expression leads to a significant accumulation of 5-hmC in the PGC genome, demethylation of the PGC genome, and activation of the expression of DNA-methylation-sensitive genes such as *DAZL*. However, the team of Ichikawa showed that *DMRT1* does not directly participate in sex determination of early PGCs (E2.5-E6.5), with female sex being established before male sex[12]. The molecular mechanisms underlying *DMRT1* regulation of PGC migration are not yet fully understood, but it is speculated that *DMRT1* may regulate the expression of genes related to cell adhesion and migration.

Gonadal sex differentiation is an important process during embryonic development. *DMRT1* plays a core regulatory role in testicular support cells, ensuring that the gonad develops into testes. During testicular formation, *DMRT1* is a hallmark gene with high expression in support cells, starting from E12.5 and being continuously expressed in the testicular cords, while this expression pattern is absent in the ovaries[13]. Research shows that *DMRT1* is crucial for fetal testicular development, as 9p deletions or point mutations can lead to complete male-to-female sex reversal in XY males, exhibiting a haploinsufficiency effect. Interestingly, unlike humans, *DMRT1* is not essential for sex determination in mouse embryos. *DMRT1*-deficient mice exhibited no abnormalities in sex determination or embryonic gonadal development, but postnatally, major defects in testicular maturation were observed, further indicating that there are differences in the expression periods of *DMRT1* between humans and mice. [7]. It is well established that vertebrates often determine sex through the XY or ZW system. The Zhang XA team used ATAC-seq and RNA-seq technologies, observing a significant upregulation of *DMRT1* gene expression and an increase in chromatin accessibility, further proving *DMRT1*'s key role in sex determination[14]. In species with ZW-type sex determination, such as birds, *DMRT1* is the main male-determining gene located on the Z chromosome, and through a dosage effect, it directly initiates testicular differentiation and suppresses ovarian pathways. Knocking out one copy of *DMRT1* in ZZ individuals results in complete sex reversal[15]. In reptiles (e.g., turtles), *DMRT1* is still located on the Z chromosome and regulates male development through dosage control, but it is not the master gene for sex determination; instead, it is a key downstream factor that maintains the testicular

pathway[16]. In contrast, in XY-type sex determination systems, *DMRT1* is not located on the sex chromosomes and is not the upstream master switch for sex determination; rather, it is a downstream essential factor responsible for postnatal and adult sex maintenance and preventing gonadal reprogramming[17].

Zhong ZW's team first established a testicular cell line (TBTc) from the double-spot fish, where they conditionally knocked out *dmrt1*. This led to a significant decrease in the expression of male-specific genes such as *sox9*, and *cyp11a*, while female-specific genes like *foxl2*, *sox3*, and *cyp19a* were upregulated, proving that *dmrt1* acts upstream, regulating testicular development by adjusting target gene expression levels[18]. *SRY*, *DMRT1*, and *SOX9* form the core regulatory axis for male sex determination and testicular development. *DMRT1*, located downstream of *SRY*, functions as a transcription factor that opens chromatin and assists *SRY* in activating *SOX9*. The two later form a complex to maintain the male fate of support cells and suppress female differentiation pathways. *DMRT1* can also bind to closed chromatin to allow *SOX9* to bind to open regions[4]. Dujardin's team constructed an XY *DMRT1*-/- conditional knockout fetal rabbit model and found that even with normal high expression of *SRY*, *SOX9* could not be activated, leading to complete gonadal differentiation into ovaries. This provides direct evidence of the *SRY*-dependent role of *DMRT1* in activating *SOX9*[19]. Massilva and others, through ChIP-seq studies on fetal testicular tissue from mice and cows, confirmed that *SOX9* binding regions are highly enriched for *DMRT1* motifs and that both proteins bind together in vivo to co-regulate testicular differentiation genes like *AMH* and *DHH*, while suppressing female genes like *FOXL2* and *WNT4*[20]. In non-rodent mammals, such as humans and rabbits, *DMRT1* is an essential factor for *SOX9* activation, and its loss causes XY sex reversal. In mice, the *Dmrt1* gene does not participate in early embryonic *SOX9* activation but plays a crucial role in maintaining male sex after birth[20, 21]. The *SRY-DMRT1-SOX9* pathway is the core regulatory module for the entire process of testicular development. The *AMH* gene has a critical role in inducing Müllerian duct regression, and it is a key male-promoting factor for sex determination. Kaitlyn's team verified that in zebrafish, *dmrt1* positively activates *amh* transcription and suppresses female-specific genes like *foxl2*, initiating and maintaining the male fate. *AMH* is a direct downstream target gene of *DMRT1*[22]. In 2025, Linda's team elucidated that, unlike placental mammals, monotremes do not have the *SRY* gene. The Y-chromosome-specific *AMHY* is the master male sex-determining gene and the first gene in mammals to function as a gender determination switch. *DMRT1* can directly bind to the *AMHY* promoter to activate its transcription, further upregulating *SOX9* expression. This process differs completely from the classic "*SRY-SOX9-AMH*" sequence found in placental mammals[23]. Interestingly, while *DMRT1* is known to positively activate *AMH*, studies have shown that in chicken embryos, *DMRT1* is a necessary transcription factor for the early formation and morphogenesis of the Müllerian ducts in both male and female sexes, positively regulating *WNT4* to maintain normal mesodermal layer formation[8]. This further highlights the complexity and temporal specificity of *DMRT1*'s functional regulation.

2.2. Maintenance and Renewal of Spermatogonial Stem Cells (SSCs)

Spermatogonial stem cells (SSCs) maintain spermatogenesis throughout adulthood by balancing self-renewal and differentiation. The *DMRT1* gene plays an important role in the maintenance and self-renewal of SSCs[24]. During the neonatal to pubertal stages, *DMRT1* is highly expressed in normal SSCs, significantly higher than in somatic cells and differentiated germ cells, where it helps maintain SSC stemness and inhibits the initiation of meiosis. After entering meiosis, *DMRT1* expression quickly decreases[25]. In 2025, Iris et al. confirm for the first time in large animals that *DMRT1* has a dosage-dependent effect (haploinsufficiency). They observed that in the *DMRT1*+/- model, SSC migration was delayed, and meiosis was prematurely initiated, ultimately leading to oligospermia and azoospermia[25]. This study

demonstrated that *DMRT1* expression in SSCs follows a precise regulatory pathway, with its levels decreasing gradually as cells differentiate. Cui N et al. found that *DMRT1* expression can be regulated by a miR-224–*DMRT1*–WNT/β-catenin feedback loop, which helps maintain SSC homeostasis. miR-224 directly binds to the 3'-UTR of *DMRT1*, inhibiting its expression. On the other hand, *DMRT1* significantly increases the mRNA and protein levels of β-catenin, activating the WNT pathway, which in turn promotes miR-224 transcription, thus forming a regulatory loop for *DMRT1*[26].

Researchers have identified molecules that work in concert with *DMRT1* in the maintenance and renewal of spermatogonial stem cells (SSCs). PLZF is a direct downstream molecule in the *DMRT1* pathway. Immunofluorescence (IF) analysis of *DMRT1*-mutant testes revealed that the expression of *PLZF* in *DMRT1*-negative spermatogonia was significantly reduced[27]. *DMRT1* co-localizes with *PLZF* in undifferentiated spermatogonia (SSCs)[4], participating in the transcriptional program of spermatogonial differentiation. PLZF primarily binds to the promoter regions of target genes[24]. Scientists have also identified upstream molecules in the *DMRT1* signaling pathway in SSCs. Kun Tan and colleagues found that Rhox10 deletion significantly downregulated both *DMRT1* and *PLZF*.Further experiments demonstrated that RHOX10 protein directly binds to the *DMRT1* promoter sequence, cooperatively drive the differentiation of pre-spermatogonia into SSCs and promoting SSC establishment and self-renewal[28]. It is also hypothesized that *DMRT1* interacts with genes such as *MAPK14*, *MAPK7*, and *BCL6B* in SSC maintenance and renewal, but this still needs further verification[29]. Conversely, PLZF directly inhibits *DMRT1* gene transcription. It binds directly to the *DMRT1* promoter region, preventing premature differentiation of spermatogonial stem cells, thereby forming a negative feedback regulatory loop between *DMRT1* and *PLZF* to sustain SSC self-renewal[30]. The authors also discovered that *PLZF* and *DMRT1* directly bind and inhibit the key switch gene *Stra8*, which initiates meiosis. This combined action helps maintain the undifferentiated state of stem cells.Several research teams have also confirmed through ChIP-seq/ChIP-qPCR techniques that *DMRT1* directly binds to the *Stra8* promoter and inhibits its transcription, thereby maintaining the stem cell state and preventing the premature initiation of meiosis[31, 32].

It is well established that the growth factor GDNF promotes SSC self-renewal in the testes through paracrine signaling via the Src and Ras pathways, playing a crucial role in the maintenance, growth, regulation, and signaling pathways of SSCs[33, 34]. As research continues to progress, scientists have also discovered the interaction between *DMRT1* and GDNF in SSCs. Yoshifumi's team found that in *cdc42* knockout mice, the expression of *DMRT1* and *SOX9* was significantly reduced, accompanied by a decrease in GDNF transcription and secretion. They proposed another signaling interaction pathway in SSCs: Cdc42 regulates the nuclear localization of MAPK1/3 through PAFAH1B1, which in turn upregulates the expression of *DMRT1* and *SOX9* in supporting cells, promoting GDNF secretion, and ultimately maintaining SSC self-renewal, localization, and sperm production stability[35]. However, this experiment did not directly explore the relationship between *DMRT1* and GDNF by knocking out or knocking down *DMRT1*, and further experiments are needed to strengthen the rigor of the findings. This also raises the question of whether other types of growth factors (e.g., EGF, IGF1, FGF2, LIF) are involved in the regulatory process of *DMRT1* in SSCs, which remains to be confirmed through further studies. Recent research has indicated that in normal male fish, the key growth axis factor IGF1 and the key sex differentiation factor *DMRT1* exhibit synergistic high expression, supporting both growth and spermatogenesis[36]. Both are also inhibited by estrogen (E_2), antagonizing male development and promoting feminization[37]. It is reasonable to speculate that IGF1 may act as an upstream signal, positively supporting *DMRT1*-mediated male differentiation, although this hypothesis still needs further validation. Meanwhile, William and colleagues found that in adult mouse and human spermatogonial

stem cells (SSCs), FGF2 and GDNF are secreted by supporting cells and cooperate through the FGFR1 and RET/GFRα1 pathways to maintain SSC self-renewal and inhibit differentiation. However, no interaction between FGF2 and *DMRT1* has been identified so far[38]. *DMRT1* plays a crucial role in initiating spermatogonial differentiation[10]. Experiments have shown that conditional knockout of *DMRT1* leads to a reduction in the expression of the differentiation key factor (SOHLH1) and an abnormal increase in the expression of meiotic initiation factor (*STRA8*). Undifferentiated spermatogonia bypass the mitotic amplification stage and directly enter meiosis prematurely, leading to severe spermatogenesis defects and reduced mature sperm counts[10].

2.3. Initiation and Regulation of Meiosis

DMRT1 is highly expressed only in mitotic spermatogonia (SSC, undifferentiated/ differentiated spermatogonia), and is silenced when pre-leptotene spermatocytes initiate meiosis, thus not entering the meiotic phase to exert its function. Zhang T and colleagues found that in *Dmrt1*-/- mice, spermatogonia bypassed the normal mitotic amplification stage and directly entered meiosis prematurely from A1 spermatogonia.[10]. Iris and colleagues demonstrated that insufficient *DMRT1* disrupted the meiotic process starting from puberty and worsened with age[25]. The role of *DMRT1* in meiosis continues to be explored. It has been confirmed that *DMRT1* is involved in three core pathways during meiosis[10]. During the meiotic process of spermatogonia, Tadashi and colleagues demonstrated that *β-TrCP* facilitates *DMRT1* ubiquitination and degradation via the ubiquitin-proteasome pathway, dissociating it from the *Stra8* promoter, thus relieving the inhibition and allowing meiosis to initiate[39]. Interestingly, *DMRT1* exhibits sex dimorphism during meiosis. Feng's team found *DMRT1* inhibits *Stra8* and prevents the initiation of meiosis in postnatal testes[32]. The normal progression of spermatogonial meiosis is the result of the coordinated action of multiple genes. Ren F and colleagues determined the expression timing and characteristics of *DMRT1* and other genes. *DMRT1* expression was significantly upregulated at the initiation stage of spermatogenesis (MII) and the regression stage (MVI), but significantly downregulated at the meiotic developmental stage (MIII) [40]. While no direct interaction between *DMRT1* and these genes has been identified now.

2.4. Spermatogenesis and Maturation

Spermatogenesis involves spermatogonia, primary and secondary spermatocytes, round spermatids, and mature sperm[41]. *DMRT1* has been repeatedly shown to play a critical role in sperm morphology changes. Li TT and colleagues found that in small-tailed cold sheep, *DMRT1* was strongly expressed in the transition from round spermatids to elongating spermatids. It directly participates in key processes such as sperm morphology remodeling, nuclear condensation, and elongation. *DMRT1* was not expressed in mature sperm[42]. However, when using roosters as the research subject, researchers found that the higher the expression of *DMRT1* in rooster sperm, the poorer the sperm deformation and maturation, with incomplete nuclear remodeling and histone replacement, smaller testis weight, and narrower seminiferous tubule diameter. This suggests that *DMRT1* may be detrimental to the completion of key steps in sperm elongation and nuclear condensation in spermatocytes[43]. It can be speculated that *DMRT1* plays an opposite role in species with XY and ZW sex determination systems.

The key genes involved in the regulation of sperm morphology by *DMRT1* remain to be fully discovered. *Tnp1* is a core gene in chromatin remodeling during sperm morphology, and is responsible for histone replacement with protamines[44]. Ran LX and colleagues found that *DMRT1* is an upstream transcriptional regulator of *Tnp1*, positively regulating its expression and jointly controlling sperm morphology. However, direct experimental verification is still lacking[45]. Other genes such as *Spag6*, *Prm*, and *CatSper* are also considered to be highly

correlated with *DMRT1* in sperm morphology. *Spag6* is a core protein of the sperm tail axoneme structure, and its absence causes tail structure abnormalities, sperm deformities, and loss of motility. Pan ZJ and colleagues confirmed that *DMRT1* and *Spag6* are specifically highly expressed in males and that their expression decreases under estradiol (E2) treatment[46]. It can be speculated that *DMRT1*, as an upstream male-determining transcription factor, positively regulates *Spag6* expression, promoting axoneme assembly and sperm maturation.

Multiple regulatory pathways are involved in maintaining the sperm maturation process, with the SPRY1–NF-κB pathway being one of the important ones. Zhang MF and colleagues demonstrated through ChIP-seq experiments that *DMRT1* can directly bind to the *Spry1* promoter region, positively regulating its expression, with a dose-dependent effect[47]. Further, they confirmed via Co-IP that SPRY1 directly interacts with NF-κB1 (p105), inhibiting the nuclear translocation of p65, thus negatively regulating NF-κB inflammatory signaling and inhibiting downstream inflammatory factors such as TNF-α, IL-1β, and IL-6. They concluded that *DMRT1* directly interacts with the SPRY1–NF-κB pathway to regulate the testicular microenvironment, protect the blood-testis barrier (BTB), maintain immune homeostasis in the testes, and ensure the normal progression of spermatogenesis and sperm maturation[47]. *DMRT1* plays a crucial role in the clearance and apoptosis of abnormal germ cells, ensuring sperm quality. Anthony and colleagues found that *DMRT1* directly binds to the *Sox2* gene regulatory region, directly inhibiting its transcription and ensuring that germ cells exit pluripotency and enter the spermatogenesis program[48]. Alexander and colleagues further discovered that *Sox2* directly inhibits *DMRT1*. After high expression of *SOX2*, cells acquire pluripotency and can differentiate into teratomas and yolk sac tumors[2]. Lin QH and colleagues found that in *DMRT1*-mutant testes, the number of TUNEL-positive cells was significantly increased, with the average number of apoptotic cells about four times that in wild-type testes, indicating that apoptosis was abnormally increased in *DMRT1*-mutant testes[49]. Sun X and colleagues confirmed that in hen follicles, *DMRT1* maintains follicle survival and development by inhibiting the Caspase-3 apoptosis pathway, and has a positive correlation with the *BCL-2* gene, supporting follicle survival[50]. Liu YX and colleagues found that PVC microplastics significantly downregulated *DMRT1* expression, accompanied by a significant upregulation of *Bax* expression and downregulation of *Bcl-2* expression[51].

3. Discussions

DMRT1, a highly conserved core transcription factor in vertebrate evolution, plays an irreplaceable role in male sex determination and germ cell fate regulation. It is essential throughout the entire process of male gonadal development, germ cell differentiation, initiation and maintenance of spermatogenesis[3]. Its core functions span the full cycle of male reproductive development. During embryonic development, *DMRT1* is involved in the directed differentiation of primordial germ cells and the establishment of male gonadal cell programs, ensuring gonadal development towards testes by repressing female differentiation genes like *foxl2* and *wnt4*, while activating male pathway genes such as *sox9* and *amh*[4, 18, 20, 22]. In the spermatogonial stem cell (SSC) stage, *DMRT1* directly binds to and represses the meiotic initiation gene *stra8*, acting synergistically with stemness factors like *plzf* to maintain stem cell homeostasis and prevent premature differentiation[8, 24, 30-32]. In meiotic regulation, *DMRT1* ensures orderly meiotic initiation and progression by regulating the retinoic acid (RA) signaling pathway, ubiquitin-mediated degradation pathways, and downstream genes related to synapsis and recombination[10, 32, 39, 40]. During spermatogenesis, it participates in critical processes like sperm shape transformation, chromatin remodeling, and flagellum assembly, while maintaining testicular immune

homeostasis and blood-testis barrier integrity via the *spry1-nf-κb* pathway, ultimately ensuring normal sperm production[5, 42, 45, 47]. At the molecular level, the function of *DMRT1* relies on its DM domain DNA-binding activity, which is regulated through a multi-layered network involving dosage-dependent effects[25, 47, 52], epigenetic modifications (DNA methylation, histone modifications)[43, 53], post-transcriptional regulation via miRNA[26], and post-translational modifications (e.g., ubiquitination)[39].

Although significant progress has been made in the study of *DMRT1*, many critical scientific issues remain unresolved. Firstly, the specific regulatory networks of *DMRT1* in different species, cell types, and developmental stages are not yet fully elucidated, particularly the direct target genes and epigenetic mechanisms of *DMRT1* in human germ cells. A systematic comparison of functional conservation and differences between humans and model organisms is still required. At the same time, the dose-effect and associated epigenetic markers and signaling pathways by which environmental factors interfere with *DMRT1* and reproductive development need further systematic validation. Regarding reproductive diseases, clinical translational research on *DMRT1* is lagging behind, and diagnostic markers, intervention targets, and therapeutic strategies for human infertility and gonadal development disorders have not yet been established. Future research should focus on cross-species comparative studies using technologies like single-cell sequencing and gene editing to clarify the functional conservation and species-specific roles of *DMRT1* in different vertebrates. In particular, a precise analysis of the spatial-temporal specificity of *DMRT1* function in human germ cells and supporting cells, as well as its downstream regulatory networks, will provide a more reliable theoretical basis for studying the mechanisms of human reproductive diseases. Clinically, there is a need to further explore *DMRT1* as a core marker for early diagnosis and to develop targeted reproductive protection and disease intervention strategies. Additionally, more research is needed on the impact of environmental factors on reproductive health. Continuous in-depth exploration of the *DMRT1* regulatory network will enhance the foundational theoretical system of male reproductive development, offering new insights and scientific support for the prevention and treatment of male infertility, gonadal development disorders, and reproductive tumors.

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