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RESEARCH HIGHLIGHT

DMRT1 at the border between mitosis and meiosis

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In males, sperm production begins at puberty and lasts throughout reproductive life. This implies that a population of germ stem cells/progenitors must be maintained, amplified and differentiate throughout life to enable the continuous supply of sperm. Spermatogenesis is indeed divided into three phases: a proliferative phase, a meiotic phase and a postmeiotic spermiogenic phase. In the proliferative phase, undifferentiated spermatogonia stem cells are divided to maintain the principle undifferentiated stem cell population on the one hand, and on the other hand to amplify and differentiate a fraction of the spermatogonia into primary spermatocytes. In the meiotic phase, primary spermatocytes undergo two reductive divisions to give haploid round spermatids which in turn, differentiate to elongated spermatids during the spermiogenic phase. The entire spermatogenic process occurs in the seminiferous tubules of the testis, where the differentiating germ cells are actually isolated from environmental signals. Sertoli cells, the only somatic cells within the tubules, can respond to environmental signals, such as hormones, and thus transmit the signals to the developing germ cells as discrete expression products that nurture them and support their differentiation. Some of these factors are expressed in a cyclical manner which is coordinated with the spermatogenic cycle and the developmental stage of the cells that associate with the Sertoli cells.

One of the most intriguing questions in developmental and reproductive biology is what regulates the transition from the proliferative phase to the meiotic phase during spermatogenesis. It has recently become evident that the switch from mitosis to meiosis requires retinoic acid (RA). Vitamin A (the precursor of RA) depletion arrests spermatogonia prior to differentiation. RA activates factors, such as STRA8, which are essential for entry into meiosis, and RA drives spermatogonia in culture to enter meiosis. Nonetheless, *in vivo*, only fully differentiated spermatogonia (type B), and not neighboring undifferentiated spermatogonia, enter meiosis in response to RA, suggesting the existence of different levels of RA responsiveness. What, then, regulates this RA responsiveness?

A paper by Matson *et al.*,[1] published in the October 19 (2010) issue of '*Developmental cell*', points at the Doublesex homolog Doublesex- and mab-3-related-transcription factor 1 (DMRT1) as the 'gatekeeper' that prevents uncontrolled entry of spermatogonia to meiosis. DMRT1 is an evolutionary conserved gonad-specific transcription factor that is essential for embryonic development of germ and Sertoli cells. Using GFRA1 and PLZF as markers for immunofluorescence colocalization, these authors showed that in adult testis DMRT1 is highly expressed in undifferentiated spermatogonia, it is less abundantly expressed in c-KIT positive differentiating spermatogonia, and it is absent from preleptotene spermatocytes or other meiotic or postmeiotic cells. DMRT1 expression in Sertoli cells was stable. To specifically evaluate the role of DMRT1 in adult spermatogonia, *Dmrt1* conditional mutants were created (*Dmrt1*$^{flox/flox}$ and *Ngn3-cre*) in which the cre recombinase is expressed under the regulation of the undifferentiated spermatogonial-specific promoter *Ngn3*, thereby deleting *Dmrt1* only in undifferentiated spermatogonia. The testes of the mutant males were smaller, and contained fewer germ cells, compared to wild-type (WT) animals, with no DMRT1, as expected. DMRT1 in Sertoli cells was unaffected. Analysis of mutant testis sections revealed that all tubules contained undifferentiated spermatogonia (E-cadherin-positive) which strongly expressed STRA8, a protein normally characterizing preleptotene spermatocytes entering meiosis. Moreover, all tubules contained STRA8 expressing cells that were positive for BrdU incorporation, indicating that the loss of DMRT1 abrogated the differentiation program of spermatogonia in a way that proliferating spermatogonia precociously entered meiosis. This conclusion was further verified by the finding that differentiating spermatogonia (c-KIT-positive) were significantly depleted in mutants. Similar results were obtained in an additional conditional mutant, made using a tamoxifen-inducible cre transgene, where tamoxifen injection activates the cre recombinase, thus deleting *Dmrt1* from spermatogonia. In this case, DMRT1 loss from spermatogonia caused significant depletion of the spermatogonial population and ectopic appearance of meiotic cells, within a week. DMRT1 expression in Sertoli cells was again unaffected due to increased stability of the protein in these cells. These results imply that the amplification divisions of the spermatogonia were bypassed, explaining why germ cell numbers were severely reduced in the mutants. Notably, the remaining germ cells entered meiosis and differentiated to haploid spermatids normally.

Next, Matson *et al.* asked whether the uncontrolled initiation of meiosis in mutant spermatogonia requires RA and STRA8 induction. To address this issue, they subjected mice (WT and mutants) to vitamin A depletion and found that in all cases cells were arrested in the spermatogonia stage, with no STRA8, not even in mutants. However, while in WT, only undifferentiated spermatogonia were observed, in mutants they could find SYCP3-positive cells indicating an arrest at a more differentiated premeiotic stage. Likewise, upon resupplementation of vitamin A, it took 6 days for leptotene meiotic cells to appear in mutant testes, whereas in WT 9–10 days were required. The authors concluded that RA and STRA8 are indeed required for entering meiosis in both normal and *Dmrt1* mutants, but mutant spermatogonia

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are arrested, under vitamin A depletion conditions, at a later stage closer to meiosis.

How, then, DMRT1 inhibits early RA-dependent entry of spermatogonia to meiosis? Given that DMRT1 and STRA8 are reciprocally expressed in B-spermatogonia and preleptotene spermatocytes, and considering the fact that loss of DMRT1 activates expression of STRA8 in spermatogonia, the authors reasoned that DMRT1 might directly inhibit STRA8 expression. Using both quantitative chromatin immunoprecipitation (qChIP) and ChIP array (ChIP-chip), the authors showed that in spermatogonia (but not in Sertoli cells) DMRT1 indeed binds the Stra8 proximal promoter. DMRT1 binding is between two RA response elements which mediate RA-dependent expression, thereby inhibiting the RA driven transcription. To test whether DMRT1 can repress other RA-dependent factors in spermatogonia, a reporter transgene, expressing β-galactosidase (βGAL) under the regulation of three RA response elements, was tested in *Dmrt1* mutants versus control. Low βGAL expression was found in control spermatogonia, whereas higher expression levels were evident in mutant spermatogonia. No binding of DMRT1 to the vicinity of the RA response elements of the transgene (or elsewhere) was detected by qChIP, suggesting that the regulation of the reporter by DMRT1 is indirect. Likewise, it was found that while transcription of the RA-dependent transcriptional stimulator, *Crabp2*, was upregulated and that of the retinoid response inhibitors, *Tbx1 and Cyp26a1*, was downregulated in mutant testis, no binding of DMRT1 to their promoter region was seen. These results led to the conclusion that DMRT1 can prevent entry into meiosis during spermatogonial differentiation, by directly blocking RA-dependent expression of STRA8 and by indirectly inhibiting other RA transcriptional activities.

Finally, Matson *et al.* present data suggesting that DMRT1 directly activates expression of SOHLH1, an essential transcription factor for spermatogonial development. They also show that loss of DMRT1 in germ cells interferes with the cyclical expression of Sertoli factors.

In conclusion, this report points at DMRT1 as an important component of the pathway which regulates the switch between the proliferative phase of spermatogonia and the meiotic phase. DMRT1 blocks meiosis and ensures the completion of the spermatogenic differentiation program by directly and indirectly suppressing RA signaling pathways which are essential for the onset of meiosis. In parallel, DMRT1 also activates factors essential for spermatogonial differentiation. This work, however, also opens new questions such as: How is DMRT1 regulated during the mitosis/meiosis switch? What role does DMRT1 play in female meiosis? How does DMRT1 interfere with the crosstalk between germ cells and Sertoli cells? These questions, and others, must await further investigation.

1 Matson CK, Murphy MW, Griswold MD, Yoshida S, Bardwell VJ *et al*. The mammalian Doublesex homolog DMRT1 is a transcriptional gatekeeper that controls the mitosis versus meiosis decision in male germ cells. *Dev Cell* 2010; **19**: 612–24.