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Human PIWI (HIWI) is an azoospermia factor

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Transposable elements (TEs) make up nearly half of the mammalian genome. Recent probabilistic re-annotation of genome sequences indicated that as much as two-thirds or more of the human genome may be derived from TEs (de Koning et al., 2011). Their ability to mobilize in the genome represents a constant threat to the host by creating insertion mutations, which may lead to progressive deterioration of genetic information. To counteract this threat, host cells have evolved adaptive control mechanisms to repress the activity of TEs. One such mechanism is the PIWI-piRNA pathway conserved in animals, from *C. elegans* to humans (Iwasaki et al., 2015). The pathway is a germline-specific mechanism required to maintain genome integrity. In this pathway, piRNAs (short for PIWI-interacting RNAs) with 25-35 nucleotides (nt) in length form effector complexes or RNA-induced silencing complexes (RISCs) with PIWI clade proteins of Argonautes to trigger multiple forms of sequence-specific gene silencing by guiding RISCs to target RNAs by means of Watson-Crick base-pairing. piRNA-loaded RISCs or piRISCs normally repress TEs either by cleaving their transcripts in the cytoplasm or by inducing heterochromatin formation at their insertion sites of the genome.

The PIWI-piRNA pathway in mammals has been studied mostly in mice (Iwasaki et al., 2015; Pillai and Chuma, 2012). Mice express three distinct *Piwi* genes: *Miwi* (*Piwil1*), *Miwi2* (*Piwil4*), and *Mili* (*Piwil2*). They are expressed at different stages during spermatogenesis in the testis, but their expression is detected only weakly, if any, in female germ cells. Consistent with their expression patterns, deficiency in each of mouse *Piwi* genes leads to the activation of TEs in the testis, and spermatogenesis is arrested at the specific stage, leading to male sterility. However, *Piwi*-deficient female mice do not show any discernible phenotype. Human *PIWI* genes are also highly expressed in the testis (Sasaki et al., 2003), which, together with the mouse *Piwi*-deficient phenotype, has led to assumptions that mutations in human *PIWI* genes may well cause male sterility. Indeed, recently in *Cell*, Gou et al. (2017) reported germline mutations in human *HIWI* (*PIWIL1*), a human ortholog of mouse *Miwi*, in patients with azoospermia.

Mouse *Miwi* expression commences only during meiosis in pachytene spermatocytes, which is accompanied by pachytene piRNAs, and peaks in the round spermatids, but its expression starts to decline in elongating spermatids and is completely eliminated in mature sperms (Iwasaki et al., 2015; Pillai and Chuma, 2012). Previously, the same authors provided evidence suggesting that the MIWI-piRISC is a target of ubiqitination by the multisubunit E3 ubiqutin ligase anaphase-promoting complex/cyclosome (APC/C) that leads to subsequent 26S proteasome-mediated degradation (Zhao et al., 2013). In this process, piRNAs appear to play an important role by enhancing the interaction of MIWI protein with an APC/C substrate-binding subunit. Amazingly, APC/C-mediated MIWI-piRISC destruction occurs only in late spermatids, which is accompanied with the clearance of associated piRNAs from spermatids, and the proper removal of the piRISC appears to be required for the successful maturation of spermatids to sperm.

As a member of the Argonaute family, PIWI proteins are characterized by four signature domains (N, PAZ, MID, and PIWI), and that the PIWI domain adopts an RNase H fold,

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which is responsible for target cleavage, while the 5′ and 3′ ends of the piRNA are anchored within the MID-PIWI interface and the PAZ domains, respectively (Matsumoto et al., 2016). A destruction box (D-box), a signature element of APC/C substrates, is present in the N domain of MIWI, which triggers APC/C binding and the ubiqutination of specific lysine residues in MIWI (Zhao et al., 2013). The D-box is conserved among vertebrate MIWI orthologs, including human HIWI. This prompted the authors to embark on a screening effort to sequence the D-box region of the *HIWI* gene in a group of 413 patients with idiopathic azoospermia (Guo et al., 2017). Guo et al., identified specific D-box mutations in three patients (0.73%) suffering from azoospermia.

Guo et al. generated knockin mice with one of the D-box mutations identified in the *Miwi* gene (referred to as $Miwi^{DB}$). All heterozygous $Miwi^{DB}$ male mice were found to be sterile, though their spermatocytes, round spermatids, and early elongating spermatids were largely unaltered. In contrast, their late condensed spermatids were strongly reduced and thus they have a reduced sperm count. These phenotypes differ from those observed in *Miwi* null mice where spermatogenesis is arrested at early round spermatids after meiosis (Deng and Lin, 2002). To test the possibility that MIWI stabilization in the late spermatids is sufficient to cause the observed phenotype, Guo et al. then generated transgenic mice expressing D-box mutant MIWI driven by the haploid spermatid-specific *Prm1* promoter (referred to as DB/Tg). DB/Tg male mice were sterile and lack elongated spermatids with extensive apoptosis in late spermatids. These findings showed that haploid-specific stabilization of MIWI in late spermatids impairs sperm maturation.

The remaining sperm in heterozygous $Miwi^{DB}$ male mice exhibited morphological defects with less condensed chromatin, suggesting defective chromatin compaction in sperm produced by the mutant mice. Proteome analysis revealed elevated levels of histones and their variants in $Miwi^{DB}$ sperm, while levels of protamines PRM1 and PRM2 in the same sample were reduced, compared with controls. This suggests that the observed defect of $Miwi^{DB}$ sperm may be

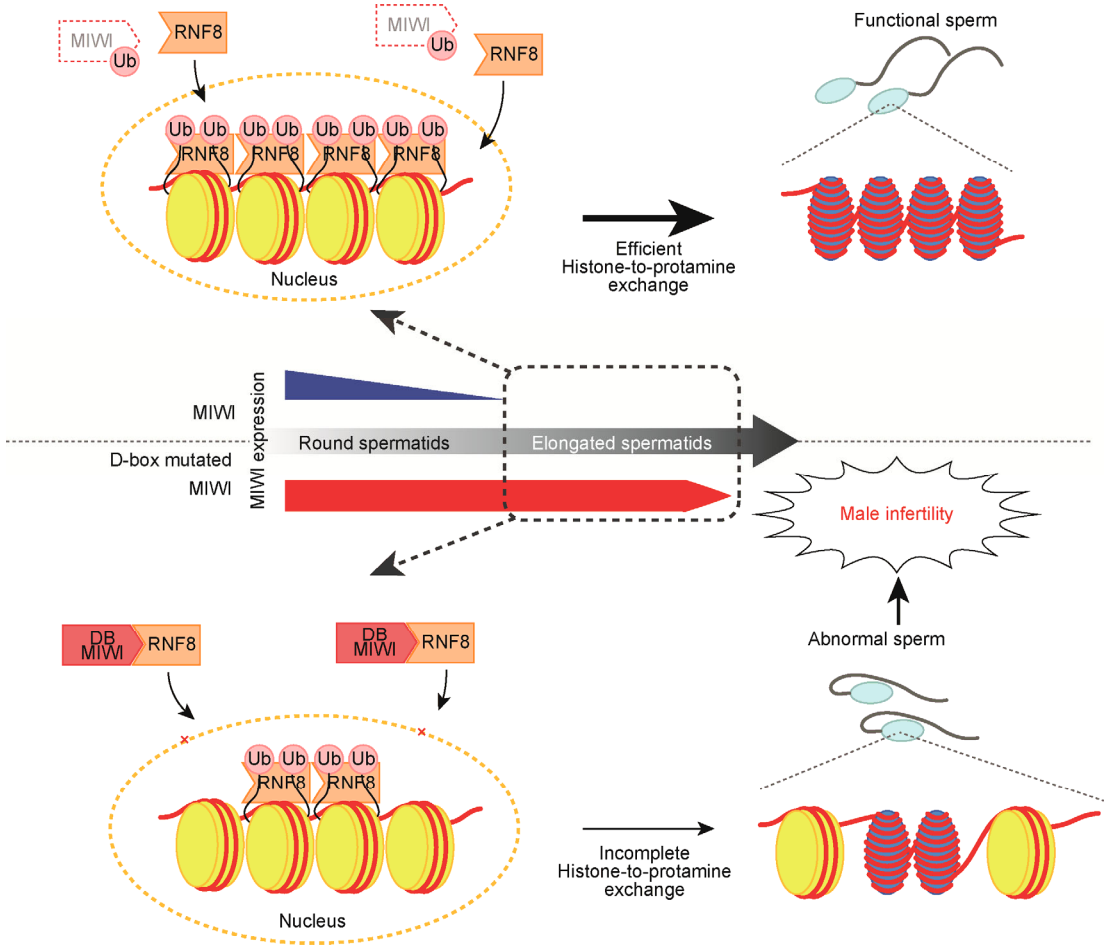

Figure 1 Life and death of MIWI in spermatogenesis. *Miwi* is expressed in spermatocytes and round spermatids to repress TEs but is eliminated in late spermatids through ubiquitination by APC/C, which is required for the maturation of speamatids to sperm. *Miwi* null mice show male infertility due to arrest in spermatogenesis at the beginning of the round spermatid stage. Gou et al. showed that mice with D-box mutated *Miwi* display spermatogenic failure at late spermatogenesis due to stabilized MIWI that ties RNF8 up in the cytoplasm to prevent histone ubiquitination, leading to incomplete histone-to-protamine exchange.

caused by an incomplete nucleosomal histone-to-protamine exchange during spermiogenesis. Proteome analysis also revealed a marked reduction of the levels of ubiquitinated H2A and H2B in late spermatids in *Miwi*^{DB} mice. Because ubiquitination of H2A and H2B by the ubiquitin E3 ligase RNF8 in elongating spermatids initiates global nucleosome removal in the mouse (Lu et al., 2010), these results suggest a model in which MIWI ubiquitination and subsequent degradation may be a trigger for histone ubiquitination and removal in late spermatids. Consistent with this model, MIWI knockdown in late spermatids of *Miwi*^{DB} mice resulted in an elevated level of ubiquitinated H2B. The notion that RNF8-deficient male mice phonocopy *Miwi*^{DB} male mice (Lu et al., 2010) also suggests that MIWI may act through RNF8 to regulate histone ubiquitination. Indeed, *in vitro* analysis indicates that MIWI is able to interact with RNF8 and interferes with its function in ubiquitination of H2B. Furthermore, immunostaining revealed that RNF8 colocalizes with MIWI in the cytoplasm of round spermatids but translocates to the nucleus of late spermatids where MIWI is eliminated. These findings together suggest a model where MIWI-piRISC sequesters RNF8 in the cytoplasm of round spermatids and its degradation promotes nuclear import of RNF8 in late spermatids, leading to histone-to-protamine exchange during spermiogenesis.

It has been known that MIWI represses the activity of TEs during meiosis from pachytene spermatocytes to the round spermatids to preserve the integrity of germline genome. The new study by Guo et al. shows that ubiquitination and subsequent elimination of MIWI in elongating spermatids is required for histone-to-protamine exchange during spermiogenesis (Figure). Thus, defects in ubiquitination of MIWI lead to male sterility. There are many future challenges. How is ubiquitination of MIWI inhibited in spermatocytes and in round spermatids? In other words, how is the inhibition lifted in late spermatids? Guo et al. sequenced only the D-box regions of *HIWI* in patients with azoospermia. However, recent studies showed that human *PIWI* genes including *HIWI* are also expressed in growing oocytes in the ovary (Roovers et al., 2015; Williams et al., 2015). This raises the possibility that *PIWI* genes in humans may be involved in oogenesis and/or early embryogenesis. It will be interesting to see whether mutations in the D-box or other regions of *HIWI* can affect female sterility.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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