Asian Journal of Andrology (2013) 15, 354–355

www.nature.com/aja

RESEARCH HIGHLIGHT

Ubiquitin meets PIWI protein

Ippei Nagamori and Toru Nakano

Asian Journal of Andrology (2013) **15,** 354–355; doi:10.1038/aja.2013.19; published online 18 March 2013

MIWI and PIWI-interacting RNA (piRNA), which binds to PIWI family members including MIWI, contribute to post-transcriptional regulation in spermiogenesis, however, their molecular functions had been unclear. Recently, Zhao and colleagues reported that loading of piRNA to MIWI leads to ubiquitin-proteasome mediated degradation in late spermatid and that this process is essential for the maturation from late spermatid to sperm. This paper casts a novel insight on the function of MIWI and the interaction between piRNA and the PIWI family member in spermatogenesis.

In the late stage of spermatogenesis (i.e., spermiogensis), chromatin becomes tightly packed by the replacement of histones to protamines *via* transition proteins. This process is essential for the capitulation of fertility and brings about not only dynamic chromatin changes but also global transcription suppression. Post-transcriptional regulation is critical for this process and involves the PIWI family of proteins. The PIWI family is a conserved gene family essential for germ cell development in many organisms ranging from *Drosophila* to humans. PIWI family proteins play crucial roles in the production of PIWI-interacting RNAs (piRNAs), a germ cell-specific subset of small RNAs that contribute to transcriptional and post-transcriptional gene suppression, presumably through the function of piRNAs.[1] Among three mouse PIWI family members (MIWI, MILI and MIWI2), MILI and MIWI2 have been reported to play essential roles in the gene silencing of retrotransposons by inducing *de novo* DNA methylation, presumably *via* piRNAs in embryonic testes. In contrast, the molecular function of MIWI, required for spermiogenesis, remains largely unknown. Recently, Zhao and colleagues showed that piRNAs induce the ubiquitination and degradation of MIWI through the APC/C proteasome pathway and that ubiquitination-mediated MIWI degradation is essential to spermiogenesis.[2] This study casts new insight on the regulation of MIWI and its role in spermiogenesis.

Zhao and colleagues first identified APC7, a component of APC/C, as one of the MIWI-binding proteins using a yeast two hybrid system.[2] This study determined that MIWI interacts not only with APC7 but also with other APC/C components. Cotransfection of chemically synthesized piRNAs, piR-1 or a testicular piRNA pool, drastically enhanced the interaction between MIWI and APC10, an APC/C substrate binding subunit, in cultured cells, whereas scrambled siRNA did not.[2] Consistent with this observation, piRNA induced the ubiquitination and degradation of MIWI in the cells, while scrambled siRNA did not. Knockdown of APC2 or APC10 and overexpression of the APC/C inhibitor Emi1 showed that the APC/C complex acts as an ubiquitin ligase for MIWI only in the presence of piRNA.

In addition, mutant MIWI (Y346/347A), which lacks the ability of piRNA loading, was not ubiquitinated or degraded even in the presence of piRNA. These data clearly demonstrate that the loading of piRNA to MIWI is required for ubiquitination-mediated degradation of the protein. The target of ubiquitination is the lysine residues at positions 330, 334 and 335, located downstream of the D-box of the MIWI protein. Finally, induction of a conformational change in MIWI by piRNA loading was revealed by an intrinsic tryptophan fluorescence assay.

These observations propose a model whereby piRNA alters the conformation of MIWI to the appropriate substrate of APC/C, and the subsequent ubiquitination-dependent degradation of MIWI in late stages of spermiogenesis is critical for the formation of spermatozoa. This model is consistent with a previous report showing that MIWI was highly abundant in pachytene spermatocytes and round spermatids but dramatically reduced in late spermatids.[3] However, why MIWI is not ubiquitinated in the earlier stages of spermatogenesis remains an enigma. One candidate for answering this question is the length of piRNAs. These authors showed that the length of piRNAs in spermatocytes was slightly shorter than that of spermatids, which are suboptimal for binding to MIWI.[2] In addition, co-immunoprecipitation and immunofluorescence analyses showed that the interaction between MIWI with APC10 in the late spermatid was stronger than that in the round spermatid and spermatocytes.[2] Although strict tests are necessary, this is a fascinating hypothesis.

We would like to emphasize the eloquent strategy of this study that used an elegant series of lentiviral gene introduction, including APC10 knockdown shRNA and a Flag-MIWI (Y346/347A) mutant into germ cells. Using this strategy, MIWI stabilization by APC10 knockdown, APC10 knockdown-induced impairment of MIWI ubiquitination and stabilization of MIWI by the introduction of the mutation for piRNA loading in the late spermatid were beautifully shown. Since an *in vitro* culture system recapitulating spermiogenesis is virtually missing, generation of knockout mice is potentially the only approach to investigate the roles of many factors involved in spermatogenesis. Therefore, many researchers have tried to establish a lentiviral vector-based gene expression/repression system in germ cells. This situation was worsened because the previously reported lentiviral vector was only able to infect Sertoli cells, but not germ cells.[4] This successful report, which includes the utility of a lentiviral vector for the study of

Department of Pathology, Medical School Osaka University, Osaka 565-0871, Japan
Correspondence: Dr T Nakano
(tnakano@patho.med.osaka-u.ac.jp)

spermatogenesis, will be welcomed by many researchers in the field.

The other big advancement of this paper is the significance of the protein turnover of MIWI and the completely unexpected role of pachytene piRNA in the process in late spermatids. piRNAs are believed to function as guides to recruit piRNA-bound PIWI proteins to complementary target RNA. A recent report indicated that MIWI-bound pachytene piRNA did not have a significant complementary target RNA.[5] Therefore, if this is true, what is the role of MIWI-bound piRNA, the concomitant by-product? The report by Zhao and colleagues appears to give a clear answer to this dilemma. Pachytene piRNA plays important roles on the conformational changes of MIWI even, without complementary target RNAs. Spermiogenic mRNAs (e.g., transition protein or protamine mRNA) have also been reported as MIWI-bound RNAs.[5] Revealing how the APC/C complex distinguishes piRNA-bound MIWI from mRNA-bound MIWI for degradation would be of interest. Another question to be answered is whether the degradation of specific proteins is a prerequisite for the last stage of spermatogenesis as well as ubiquitin-dependent MIWI protein degradation.

While many infertile male patients have been treated by intracytoplasmic sperm injection, the molecular mechanisms underlying spermatogenesis should be an important approach to consider in drug discovery for these patients. piRNA/PIWI-mediated gene expression could be a candidate.

1 Grivna ST, Beyret E, Wang Z, Lin H. A novel class of small RNAs in mouse spermatogenic cells. *Genes Dev* 2006; **20**: 1709–14.
2 Zhao S, Gou LT, Zhang M, Zu LD, Hua MM *et al*. piRNA-triggered miwi ubiquitination and removal by APC/C in late spermatogenesis. *Dev Cell* 2013; **24**: 13–25.
3 Deng W, Lin H. miwi, a murine homolog of piwi, encodes a cytoplasmic protein essential for spermatogenesis. *Dev Cell* 2002; **2**: 819–30.
4 Ikawa M, Tergaonkar V, Ogura A, Ogonuki N, Inoue K *et al*. Restoration of spermatogenesis by lentiviral gene transfer: offspring from infertile mice. *Proc Natl Acad Sci USA* 2002; **99**: 7524–9.
5 Vourekas A, Zheng Q, Alexiou P, Maragkakis M, Kirino Y *et al*. Mili and Miwi target RNA repertoire reveals piRNA biogenesis and function of Miwi in spermiogenesis. *Nat Struct Mol Biol* 2012; **19**: 773–81.