Asian Journal of Andrology (2012) 14, 513

www.nature.com/aja

RESEARCH HIGHLIGHT

Pum 1 sequesters apoptosis during spermatogenesis

W Steven Ward

Asian Journal of Andrology (2012) **14,** 513; doi:10.1038/aja.2012.35; Published online: 23 April 2012

RNA binding proteins have long been known to play a role in spermatogenesis. The laboratory of Norman Hecht was one of the leaders in this field, having characterized three RNA binding proteins that regulate mRNAs during sperm differentiation. Polypyrimidine tract-binding protein 2 (PTB2) has two functions—stabilizing mRNA transcripts in the cytoplasm and being involved in splicing.[1] Using *in vivo* cross-linking of protein to RNA, several mRNA and small RNA targets were identified in the testis.[2] Translin is another protein that both stabilizes mRNAs from a specific transcription factor, and is associated with small RNAs.[3] Perhaps the most interesting is the DNA/RNA binding protein MSY2. At low concentrations, MSY2 activates transcription, but at higher concentrations, the protein stabilizes the mRNAs that it had previously activated.[4] This plays a particularly useful role in spermiogenesis as it stabilizes and stores the transcripts of several genes that are required long after transcription has stopped. MYSY2 has also recently been shown to bind specifically to small RNAs 25–33 nt in length, suggesting a broader role for this protein than is currently known.[5]

Recently, Chen *et al.*[6] have added another protein to the known group of RNA-binding proteins that are involved in spermatogenesis. These authors had been interested in a *Drosophila* protein, Pum, which post-transcriptionally regulates several genes in the germline. In their current work, they performed a detailed study of the mouse homologue PUM1. They first showed that it is highly expressed in the testis, though other tissues had significant levels of PUM1. In the testis, immunocytochemical localization indicated that Pum 1 is expressed in the cytoplasm of spermatocytes and spermatids. Pum 1 knockout males have reduced sperm counts and reduced litter sizes, but they remain fertile. The testes of $Pum1^{-/-}$ mice show much higher levels of apoptosis in spermatogonia, and reduced levels of spermatogenesis.

The authors then used a genome wide RNP-Chip assay to identify RNAs that were specifically associated with PUM1 in the testis. They identified 3687 transcripts that represented 1527 genes. These genes were then analyzed by MetaCore to identify 11 biochemical pathways that were enriched in the 1527 genes. One of these was a pathway that regulates p53 that included nine of the genes identified as binding to PUM1. They then confirmed that all nine proteins were increased by Western blots. To confirm that PUM1 has a regulatory effect on the p53 pathway, they crossed $Pum1^{-/-}$ mice with a line that has a mutant p53 gene and showed that the apoptosis in the testis was reduced.

The authors conclude that spermatogonia are normally removed by apoptosis during spermatogenesis, but that this process must be regulated; otherwise, too many of the spermatogonia will be lost. PUM1 is a strong candidate for at least some of this regulation, and its mode of action is through mRNA binding. However, in contrast to MSY2, for example, PUM1 seems to inhibit translation of the mRNAs permanently, rather than stabilizing the mRNA for translation at its appropriate time.

As demonstrated by the results from both groups, the issue of how RNA-binding proteins regulate spermatogenesis is far from understood. It is likely that MSY2 and PTB2 play a role in regulating small RNAs, but understanding how they do this will have to evolve as the still new field of micro RNA continues to explode. For PUM1, the immediate question is how does its interaction with the other 8 pathways and 1518 genes contribute to spermatogenesis? It would also be interesting to know whether the $Pum1^{-/-}$ mice crossed with the mutant p53 mice had restored fertility. If not, this might be an important model for understanding other roles PUM1 has in male fertility. Another question is whether PUM1, like MSY2 and PTB2, also binds to non-coding small RNAs and plays a role in regulating these important cell modifiers.

This work highlights, once again, the importance of RNA-binding proteins during spermiogenesis. It is likely that we have still only scratched the surface on this important molecular process.

Department of Anatomy, Biochemistry and Physiology, Institute for Biogenesis Research, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI 96822, USA
Correspondence: Professor WS Ward (wward@hawaii.edu)

1 Black DL. Mechanisms of alternative pre-messenger RNA splicing. *Annu Rev Biochem* 2003; **72**: 291–336.
2 Xu M, Hecht NB. Polypyrimidine tract-binding protein 2 binds to selective, intronic messenger RNA and microRNA targets in the mouse testis. *Biol Reprod* 2011; **84**: 435–9.
3 Iguchi N, Tobias JW, Hecht NB. Expression profiling reveals meiotic male germ cell mRNAs that are translationally up- and down-regulated. *Proc Natl Acad Sci USA* 2006; **103**: 7712–7.
4 Yang J, Medvedev S, Reddi PP, Schultz RM, Hecht NB. The DNA/RNA-binding protein MSY2 marks specific transcripts for cytoplasmic storage in mouse male germ cells. *Proc Natl Acad Sci USA* 2005; **102**: 1513–8.
5 Xu M, Medvedev S, Yang J, Hecht NB. MIWI-independent small RNAs (MSY-RNAs) bind to the RNA-binding protein, MSY2, in male germ cells. *Proc Natl Acad Sci USA* 2009; **106**: 12371–6.
6 Chen D, Zheng W, Lin A, Uyhazi K, Zhao H *et al*. Pumilio 1 suppresses multiple activators of p53 to safeguard spermatogenesis. *Curr Biol* 2012; **22**: 420–5.