

Temporal control of protein synthesis during spermatogenesis

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Summary

During oogenesis and spermatogenesis transcription ceases prior to the differentiation of the mature cells. To complete germ cell differentiation and initiate early embryogenesis, proteins are synthesized from pre-existing mRNAs that are stored for several days. It is well established that important regulatory elements functioning in spatial localization, temporal translation or messenger RNA stability are located in the 3' untranslated region (3' UTR) of mRNAs. During mammalian spermatogenesis temporal translational regulation of the protamine 1 (*Prm1*) mRNA is dependent on a highly conserved sequence located in the distal region of its 3' UTR. The 17-nucleotide translational control element (TCE) mediates translational repression of the *Prm1* mRNA. Mutation of the TCE causes premature synthesis of protamine protein and sterility. The *Prm1* mRNA is stored as a cytoplasmic ribonucleoprotein (mRNP) particle in spermatids. Contained within the particle are several members of the Y box family of nucleic acid binding proteins. In the yeast three-hybrid system the murine Y box proteins MSY1, MSY2 and MSY4 bind in a sequence-dependent manner to a conserved region in the proximal portion of the *Prm1* 3' UTR. Sequence-specific binding by MSY4 to the Y box recognition sequence (YRS) is dependent on the highly conserved cold shock domain, possibly through the RNP1 and RNP2 motifs present within it. The Y box proteins may function as translational repressors in vivo. Alternatively, their primary function may be to protect mRNAs from degradation during their extended period of storage. Translational activation of stored mRNAs is essential for the completion of gametogenesis. Proper translational activation of the *Prm1* mRNA in elongated spermatids requires the cytoplasmic double-stranded RNA binding protein TARBP2. *Tarbp2* is expressed at low levels in many cells but is expressed at robust levels in late stage meiotic cells and in postmeiotic spermatids. Mice mutant for *Tarbp2* are defective in proper translational activation of the *Prm1* and *Prm2* mRNAs and are sterile. Current studies are designed to determine the mechanism by which proteins bound to the 3' UTR communicate with the 5' end of the message to control translational silencing and activation.

Keywords: post-transcriptional control, protamine, spermatogenesis, translational regulation

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Translational control is important during gametogenesis, as there are times when protein synthesis occurs in the absence of transcription. In amphibians, early development is dependent on stored mRNAs until the mid-blastula

transition when zygotic transcription first initiates at the 1000 cell stage (Davidson, 1986). In mice, transcription ceases at meiotic maturation in oocytes and reinitiates after fertilization in the 2-cell embryo (Hogan *et al.*, 1994). During spermatogenesis transcription ceases in the haploid spermatids at the time of chromatin condensation (Monesi, 1964). Some mRNAs are stored for more than a week before they are recruited for translation at specific times during sperm morphogenesis. Among the mRNAs that are under translational control in spermatids are protamine 1 (*Ppm1*) and 2 (*Ppm2*). The protamines mediate nuclear condensation during the terminal stages of spermatid differentiation. Translational delay of the *Ppm1* message is essential for normal spermatogenesis in mammals. Mutations that result in premature translation of the *Ppm1* mRNA cause precocious nuclear condensation and sterility (Lee *et al.*, 1995).

Like many localized or stored mRNAs, sequences responsible for the translational silencing of *Ppm1* lie within the 3' UTR of its mRNA (Braun *et al.*, 1989). Several different regions have been suggested to function in silencing; however, recent transgenic evidence suggests that a 17-nucleotide (nt) sequence, the translational control element (TCE), is essential for silencing (Zhong *et al.* 2000). The TCE is located in the distal region of the 3' UTR and is highly conserved between *Ppm1* and *Ppm2*. Mutation of the TCE in the context of the full-length *Ppm1* 3' UTR abolishes translational silencing. The binding site for a sequence-specific RNA binding complex (Fajardo *et al.*, 1994, 1997), and the Y and H boxes (Kwon & Hecht, 1993), can mediate translational repression in transgenic mice and in vitro, respectively, but none of the sites is sufficient for translational delay in its normal position within the 3' UTR in vivo.

In the female germline it has been known for several years that stored mRNAs are associated with masking proteins. In *Xenopus* eggs the major masking proteins are members of the evolutionarily well conserved family of Y box proteins (Sommerville, 1999). The *Xenopus* Y box protein FRGY2 (mRNP₃₊₄ or p54/p56) is a major component of mRNPs in oocytes (Darnbrough & Ford, 1981) and at high concentrations is able to repress translation in vitro (Bouvet & Wolffe, 1994; Ranjan *et al.*, 1993; Richter & Smith, 1984). All family members contain the highly conserved cold shock domain (CSD) that is 43% identical from bacteria to humans. Within the CSD domain are the RNA binding motifs RNP-1 and RNP-2. Y box proteins contain a variable amino-terminus, the CSD, and four alternating basic and acidic regions at the carboxyl terminus. The amino acids in the basic/acid regions are not highly conserved among family members, but the structure of alternating domains is conserved.

Thus far three genes encoding Y box proteins have been described in mammalian germ cells. MSY1, identified by heterologous antibody screening, is expressed in a broad range of tissues and appears to be orthologous to the major somatic Y box protein in *Xenopus*, FRGY1 (Tafari *et al.*,

1993). MSY2, also discovered by immunoscreening, appears to be the mouse ortholog of FRGY2 (Gu *et al.*, 1998). The third murine Y box protein, MSY4, was cloned using the yeast three-hybrid system (Davies *et al.* 2000) in a screen for a previously characterized sequence-specific RNA binding activity present in germ cells (Fajardo *et al.*, 1994). MSY4 shares the same structural organization as MSY1 and MSY2, but shows a higher degree of similarity to the human Y box protein DBPA (Coles *et al.*, 1996; Sakura *et al.*, 1988).

Y box proteins have been shown to have both sequence-specific and sequence non-specific nucleic acid binding properties in vitro. The Y box proteins were first identified as transcriptional activators that bind the Y box DNA sequence (CTGATTGGC/TC/TAA) (Deschamps *et al.*, 1992; Sakura *et al.*, 1988). Y box proteins were later shown to also bind RNA non-specifically (Murray, 1994; Tafari & Wolffe, 1993). The repeated basic and acidic regions of the *Xenopus* FRGY2 protein mediates non-specific RNA binding. Bouvet *et al.* (1995) have used an in vitro selection amplification technique, SELEX, to show that FRGY1 and FRGY2 preferentially bind the 6-nt sequence 5' ACCAUC 3'; however, specific *Xenopus* mRNAs containing this sequence have not been identified. While both the N-terminus and the C-terminus were found to contribute to the interaction with RNA, specific RNA binding mapped to the RNP motifs within the CSD. Extensive mutational analysis of the *Ppm1* Y box recognition site (YRS) in vitro and in the three-hybrid assay, strongly support the conclusion that MSY2 and MSY4 bind a specific 7-nt RNA sequence, the Y box recognition sequence (YRS) (5' UCCAUCA 3'), perhaps through the RNP1 and RNP2 motifs in its CSD. The similarity of the FRGY1/FRGY2 and MSY1/MSY2/MSY4 binding sites, and their cold shock domains, suggest the possibility that all Y box proteins bind similar but different RNA sequences. Recent evidence suggests that MSY1, MSY2 and MSY4 may all be contained in a sequence-specific RNA binding activity previously described.

In male germ cells MSY1, MSY2 and MSY4 are localized in the cytoplasm of meiotic cells and postmeiotic spermatids. MSY4 is also expressed in the ovary and is specifically localized in the cytoplasmic compartment of both preantral and antral follicles (Davies *et al.* 2000). Y box proteins sediment with translationally repressed mRNP particles in sucrose gradients. Within mRNPs Y box proteins could be directly responsible for translational silencing. Support for this notion is that a transgene deleted for the distal TCE region of the *Ppm1* 3' UTR, but containing the YRS, is translationally repressed in vivo (Fajardo *et al.*, 1997). However, in the context of the full-length *Ppm1* 3' UTR, the YRS is not sufficient for translational repression (Zhong *et al.* 2000). Base substitution mutations in the TCE cause premature translation, even with an intact YRS. An alternative possibility is that Y box proteins function in mRNA stability. Other proteins, not yet identified, may be

the true translational repressors of the *Pmm1* mRNA. The Y box proteins may associate with translationally repressed mRNAs, masking and protecting them from degradation during their long period of storage.

Completion of spermatogenesis requires the correct temporal activation of stored messages. Little is known about how activation is regulated. Because global transcription is inhibited in elongated spermatids, translational activation must occur independent of transcriptional control. Activation could be under cell-autonomous control. Alternatively, activation could be controlled by Sertoli cells and require cell-cell communication or hormonal signaling. Transgenic experiments suggest assembly of the mRNA into translationally silent mRNPs is important for activation. Mutations in the *Pmm1* 3' UTR can interfere with efficient activation of translation (Fajardo *et al.*, 1997). Similarly, a

targeted deletion of the double-stranded RNA binding protein encoded by the *Tarbp2* locus results in inefficient activation of the *Pmm1* and *Pmm2* mRNAs (Zhong *et al.*, 1999). The expression of *Tarbp2* during the time of mRNP assembly suggests TARBP2 functions in the assembly of a translationally competent mRNP.

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