seminars in **CELL & DEVELOPMENTAL BIOLOGY,** Vol 9, 1998: pp 483–489 *Article No. sr980226*

Post-transcriptional control of gene expression during spermatogenesis

Robert E. Braun

The synthesis and storage of mRNAs prior to their translation is a necessity during spermatogenesis as global transcription ceases several days prior to the completion of spermatid differentiation. Post-transcriptional control can be mediated by sequences in the 5′ and 3′ untranslated regions of mRNAs, and in some cases separate elements may regulate translational repression and translational activation. Translational repression is essential for spermatid differentiation as premature translation can lead to an arrest in spermatid differentiation and cause dominant male sterility.

Key words: Post-transcriptional / Control / Protamine / Spermatogenesis / Translational regulation

When transcription stops and translation starts

MURINE SPERMATOGENESIS INITIATES a few days after birth and continues for the duration of the reproductive life of the animal. Spermatogenesis takes approx. 35 days and consists of the mitotic proliferation of spermatogonial cells, meiosis and spermiogenesis, the haploid spermatid differentiation stage. Transcription is ongoing throughout spermatogonial proliferation, meiosis and early spermiogenesis. In fact, unlike spermatogenesis in *Drosophila*, where there is no post-meiotic transcription,[1] in the mouse there is considerable elevation in the transcriptional apparatus shortly after meiosis.[2] Transcription continues until the transition from the round to the elongating spermatid and then ceases several days prior to the completion of spermiogenesis.[3,4] The study of transcriptional activity has relied primarily on metabolic labeling studies which are limited in their sensitivity. Thus, the available data do not allow us to discern whether transcriptional silencing occurs in the early or the late elongating spermatid. Distinguishing between these two possibilities is important if one is to understand the reason for transcriptional silencing. Cessation of transcription in the early elongating spermatid (~ steps 9–10) most likely would involve developmental changes in the transcriptional apparatus, whereas transcriptional arrest in late elongating spermatids (~ steps 12–13) could be attributed to changes in chromatin structure that occur as chromosome condensation commences. Nonetheless, in both the fruit fly and in mice, there is a need for translational control due to transcriptional silencing. In *Drosophila*, transcription ceases in meiosis and in the mouse it ceases in elongating spermatids. In both cases, translational activation of stored messages is required to complete spermatid differentiation.

Chromosome condensation in mammalian spermatids is initiated by the transition proteins (TPs) and is completed by the protamines.[5] The mRNAs that encode both of these classes of proteins are under translational control. In the case of the two protamine genes, *Prm-1* and *Prm-2*, the genes are transcribed initially in step 7 spermatids, but their respective mRNAs are not translated until approx. step 13.[6–8] Translational repression of *Prm-1* and *Prm-2* occurs at a time when other mRNAs are actively translated. Thus, repression of protamine translation is selective and must be regulated.

In addition to those mRNAs that are repressed translationally until after transcription ceases in elongating spermatids, many other mRNAs are repressed translationally during meiosis and in early round spermatids, but are translated in round or elongating spermatids (for an extensive compilation of such examples, see Kleene[9]). Specific examples of such translational control include *PGK-2*,[10–12] *Tenr*[13] and *Spnr*.[14] The TENR protein is a testis-specific, RNA-binding protein that is expressed post-meiotically and is present in a lattice-like structure within the round

From the Department of Genetics, Box 357360, 1959 NE Pacific, University of Washington, Seattle, WA 98195, USA.

1084-9521/ 98/ 040483+ 08 $30.00/ 0

spermatid nucleus. SPNR is a microtubule-RNA-binding protein that associates with the manchette in elongating and elongated spermatids and may function in translational activation of stored mRNAs.[14] *PGK-2*, *Tenr* and *Spnr* are all transcribed in pachytene spermatocytes but their mRNAs are repressed translationally until spermiogenesis. It is not evident why mRNAs that are translated before the cessation of transcription are under translational control, nor is it necessary that the mechanisms controlling their translation be similar to that of the transition proteins and the protamines.

Regulatory elements in the mRNA

Translational control elements have been identified in both the 5′ and 3′ untranslated regions (UTRs) of mRNAs under translational control.[15,16] It is likely that the mechanisms of translational repression differ for 5′ UTR- vs. 3′ UTR-mediated control. In general, regulatory elements located in the 5′ UTR are more likely to function by sterically hindering the assembly or translocation of the 43S initiation complex. Control elements in the 3′ UTR also may inhibit translational initiation, but are more likely to do so by nucleating the assembly of a translationally inert mRNP, by sequestering the mRNA into a subcellular location within the cell, or by interfering with poly(A)-mediated initiation and eIF-4F (cap-binding complex) binding to the 5′ end of the message.

Translational repression of *Prm-1* mRNA is controlled by sequences in its 3′ UTR. Demonstration that the *Prm-1* 3′ UTR controls its translation was shown by using gene fusions in transgenic mice.[17] Chimeric mRNAs containing the human growth hormone coding sequences fused to the *Prm-1* 3′ UTR are repressed translationally in round and elongating spermatids and active translationally in elongated spermatids, in the same manner as the endogenous *Prm-1* mRNA. Thus, sequences in the 3′ UTR mediate both proper translational repression in round spermatids and translational activation in elongated spermatids. In the case of *PGK-2* mRNA, transgenic analysis has shown that the 5′ UTR is not sufficient for translational repression, implying that sequences elsewhere in the mRNA must contain the regulatory elements.[12]

Translational inhibition of *Prm-1* mRNA is absolutely essential for the completion of spermiogenesis.[18] Premature translation of a *Prm-1* transgene results in precocious chromosome condensation and dominant male sterility. This dominant male sterility is due to the sharing of post-meiotic gene products between spermatids.[19] Sharing of gene products is possible because of the intercellular bridges that connect cells within developing syncytial clones of spermatids.[20]

At least two separate regions of the *Prm-1* 3′ UTR are capable of repressing the translation of a reporter mRNA in transgenic mice. The regions are non-overlapping, suggesting that there are unique redundant elements capable of translational repression. The first of these regions maps to the 5′-most 37 nucleotides (nts) of the *Prm-1* 3′ UTR.[21] Within this region is a 9-nt sequence that is conserved at seven of nine positions in the *Prm-2* 3′UTR (Figure 1). The second region that is sufficient for translational repression is contained in the 3′-most 62 nts of the *Prm-1* 3′ UTR.[22] Within this region is a 17-nt sequence, which is present also in the *Prm-2* 3′ UTR, and shares identity at 14 of 17 positions (Figure 1). A second copy of the 17-nt sequence is present in the *Prm-1* 3′ UTR, nucleotides 80 and 95, but absent in the *Prm-2* 3′ UTR. Although independent transgenic analysis of both the 5′-most 37 nts and the 3′-most 62 nts of the *Prm-1* 3′ UTR has shown that each is capable of translational repression *in vivo*, neither region appears to repress translation as well as the full-length *Prm-1* 3′ UTR. Completely wild-type levels of translational repression appear to require the presence of both halves of the *Prm-1* 3′ UTR. This added stringency may be beneficial for spermiogenesis given the detrimental consequences of premature expression of PRM-1 protein on the developing spermatid.[18]

Translational activation of the *Prm-1* mRNA may require separate elements from those involved in translational repression. By analysing the expression of a reporter transgene containing the translational silencer in the first 37 nts of the *Prm-1* 3′ UTR, but lacking a portion of the 3′-most region of the 3′ UTR, Fajardo *et al.*[21] showed that the reporter mRNA was poorly activated for translation in elongated spermatids. Failure to activate translation could be due to the absence of specific sequences required for activation. Transgenes that contain the 3′-most 62 nts of the *Prm-1* 3′ UTR activate translation normally, suggesting that sequences required for activation map to that region.[22] It is not known if the same sequences that mediate repression through the 3′-most 62 nts, possibly the conserved 17-nt sequence, also mediate translational activation.

By using RNA electrophoretic mobility shift assays (EMSAs), Kwon and Hecht[23] have identified two re-

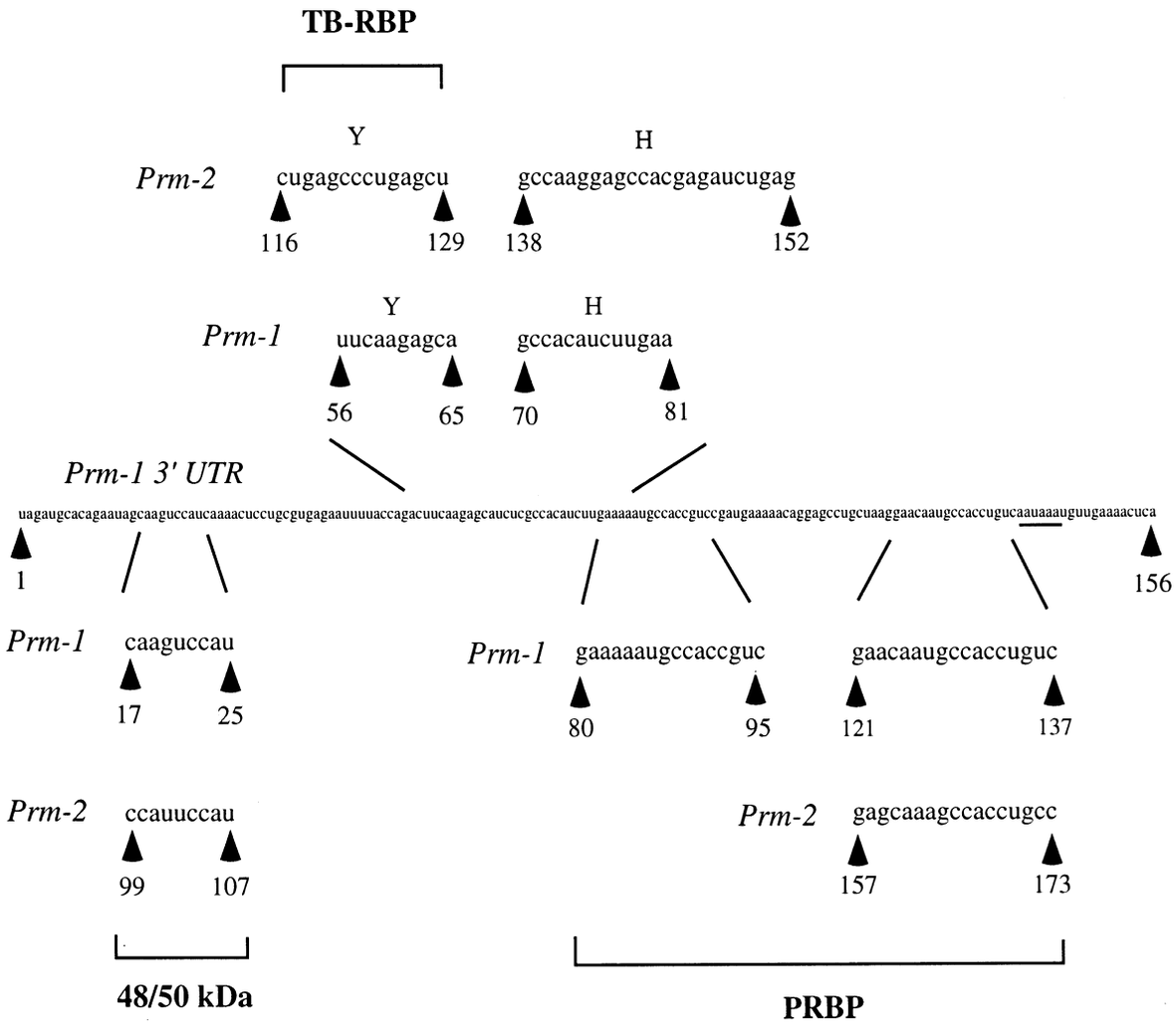

Figure 1. Translational control elements in the protamine 3′ untranslated regions. The *Prm-1* 3′ UTR is 156 nts, excluding a poly(A) tail of approx. 150 nts. Sequences in the *Prm-2* 3′ UTR that are similar to those found in *Prm-1* are listed. The regions where proteins are known to bind are marked by brackets.

gions in the *Prm-1* and *Prm-2* 3′ UTRs, referred to as the Y and H elements, that bind a protein present in testis extracts (Figure 1). The Y and H elements of *Prm-2* have been shown to mediate translational repression of a reporter mRNA in a rabbit reticulocyte lysate supplemented with a testis extract enriched for the binding factor, suggesting that the site is capable of mediating translational repression.[24] It will be important to test the Y and H elements *in vivo*, by using transgenic mice.

In *Drosophila* spermatogenesis, a family of mRNAs referred to as *Mst (3) CGP*, are synthesized in pachytene spermatocytes and translated in fully elongated spermatids.[25–27] The products of these mRNAs are thought to be components of the satellites positioned on the outside of the axoneme. Members of this family of mRNAs share a 12-nt sequence, referred to as the translational control element, or TCE, located within the first 40 nts of their 5′ UTRs. Transgenic analysis has demonstrated convincingly that the TCE is required for translational control *in vivo* and that the position of the TCE in the 5′ UTR is critical for translational repression.[28] TCE elements have been found in other *Drosophila* mRNAs as well, although their position within the 5′ UTR does not appear to be conserved as stringently as that for the

Mst (3) CGP mRNAs.[29] Similar elements in murine spermatogenic mRNAs have not been described.

The complexity of the protamine mRNA ribonucleoprotein particle

Elucidating the components of the protamine mRNA ribonucleoprotein particle (mRNP) may facilitate our understanding of the mechanism of translational control. Unfortunately, without a specific component of the protamine mRNP in hand, it is difficult to purify it from the other mRNPs present in round spermatids and to characterize its complexity. Nonetheless, it is known that the *Prm-2* mRNP sediments at less than 80 S in Nycodenz gradients[30] and that it may contain poly(A)-binding protein, PABP.[31] The presence of PABP in the protamine mRNP suggests that the 3′ UTR does not inhibit translation initiation by preventing binding of PABP to the poly(A) tail. However, the stoichiometry of PABP bound to the protamine message is unknown and may be less than optimal for mediating translation initiation.

The protamine mRNP may contain at least one Y box protein. Y box proteins are sequence-specific DNA-binding proteins and non-specific RNA-binding proteins.[32] Y box proteins of 54 and 56 kDa originally were identified as components of stored maternal mRNPs in cytoplasmic extracts prepared from *Xenopus laevis* oocytes.[30,33–37] Homologues of the *Xenopus* p54/p56 proteins have been described in mouse testicular extracts and shown to have apparent molecular weights of 48/52 kDa[38] and the gene for the 52 kDa protein has been cloned and is referred to as mouse Y box protein, MSY1.[30] The mouse p48 and p52 Y box proteins are highly enriched in the testis and have been shown to bind non-specifically to various RNAs *in vitro*, including *Prm-1*, *Prm-2*, *TP1*, *hGH* and pGem-2 RNA.[38] Although MSY1 fractionates with mRNPs in Nycodenz gradients,[30] it has not been demonstrated that p48 and p52 actually are associated with the protamine mRNAs *in vivo*.

Protamine RNA-binding proteins

Given the non-specific RNA-binding properties of the MSY1 Y box protein and the evidence that the protamine mRNPs are assembled at a time when other mRNAs are active translationally, one also would expect there to be sequence-specific, protamine RNA-binding proteins. Such proteins might initiate or direct the assembly of the protamine mRNP and thereby confer the necessary specificity required for selective translational inhibition. Several candidate protamine RNA-binding proteins have been described.

By using a combination of RNA EMSAs and UV crosslinking, Fajardo *et al* have detected a 48/50 kDa protein complex that binds to a conserved sequence in the *Prm-1* and *Prm-2* 3′ UTRs.[39] Competition assays reveal that the complex is specific for *Prm-1* and *Prm-2* mRNAs and mutational analysis and RNAse footprinting studies have been used to map the binding site to a specific region in the 5′-end of the 3′ UTRs (Figure 1). Transgenic analysis of a reporter RNA that contains the binding site for the 48/50 kDa proteins is repressed translationally in round spermatids, although repression is incomplete.[21] The gene that encodes the 48/50 kDa binding activity has been cloned from a mouse testis cDNA library by using the yeast three-hybrid system (H. Davies and R.E. Braun, unpublished). Surprisingly, the RNA-binding protein is a new member of the Y box family of RNA-binding proteins. As described above, initial studies of the Y box family of RNA-binding proteins suggested that they are components of the translational masking machinery and that they bind RNA non-specifically.[32] However, by using a method of *in vitro* selection and amplification, Bouvet *et al*[40] recently have shown that the FRG Y2 Y box protein can recognize specific target sequences. The 48/50 kDa Y box protein that binds to the *Prm-1* 3′ UTR clearly binds RNA in a sequence-specific manner as point mutations in the binding site eliminate binding in the three hybrid assay and in an EMSA (F. Giorgini, H. Davies and R.E. Braun, unpublished). It may be that other Y box proteins, previously considered to be non-specific RNA-binding proteins, also are sequence-specific RNA-binding proteins, but whose *in vivo* target RNAs are unknown.

Two regions of the *Prm-1* 3′ UTR have been shown to be able to repress the translation of a heterologous mRNA in transgenic mice. The first region maps to the first 37 nts of the 3′ UTR and contains the binding site for the 48/50 kDa Y box protein described above.[21] The second region is contained within the 3′-most 62 nt of the 3′ UTR.[22] The 48/50 kDa protein does not bind to the 62-nt region, thus there must be other translational control factors that mediate the repression through this region. One possibility is the protamine RNA-binding protein, PRBP. The *Prbp* gene was cloned in an expression

screen for cDNAs that encode *Prm-1* 3′ UTR RNA-binding proteins.[41] PRBP is a 40 kDa protein that contains two copies of a well conserved double-stranded RNA-binding motif.[42,43] PRBP is localized to the cytoplasm of pachytene spermatoctyes and round spermatids consistent with it mediating translational control. In an RNA EMSA, bacterially expressed PRBP binds to a region of secondary structure in the 3′-most region of the *Prm-1* 3′ UTR (Figure 1). PRBP is capable of inhibiting translation *in vitro*, but it is not specific to mRNAs containing the *Prm-1* 3′ UTR. Despite the fact the PRBP binds to a region of the 3′ UTR shown to be capable of inhibiting translation *in vivo*, its classification as a general double-stranded RNA-binding protein weakens the potential for its involvement as a specific mediator of *Prm-1* translational inhibition. Demonstration that PRBP functions as a translational repressor of *Prm-1* awaits mutation of the gene *in vivo*.

The Y and H elements in the *Prm-1* and *Prm-2* 3′ UTRs interact with a testis-brain, RNA-binding protein, TB-RBP (Figure 1). The gene encoding TB-RBP has been cloned and is the mouse homologue of the human translin protein.[44] Translin binds single-stranded DNA *in vitro* and has been proposed to be associated with regions of breakpoint junctions of chromosomal translocations in some human malignant lymphoid cells.[45] The potential involvement of TB-RBP in DNA-associated events in the nucleus and RNA-associated functions in the cytoplasm, may be regulated by post-translational modifications.[44] The sequence-specific binding of TB-RBP to the H and Y elements and the ability of TB-RBP-enriched fractions to selectively inhibit translation *in vitro*, suggest that it could be a regulator of *Prm-2* translation *in vivo*. Further support for such a function would be the demonstration that the H and Y elements can repress the translation of a heterologous mRNA in transgenic mice and that mutation of TB-RBP leads to premature translation *in vivo*.

Activation of translationally repressed mRNAs

Translational activation requires that the mRNA be mobilized from repressed mRNPs onto polysomes in elongated spermatids. By some unknown mechanism the repression apparatus must be modified to release the mRNA for translation. Synthesis of a translational activator, like a protein kinase or phosphatase that modifies the mRNP, is a possibility. The RNA-binding properties of TB-RBP appear to be coupled to its phosphorylation status[23,24] and are consistent with such a model. Of course, synthesis of a translational activator of the protamine mRNAs only tells us how activation of protamine translation is achieved. It does not tell us how synthesis of the activator is regulated in the absence of new transcription. The ultimate question is what is the initial trigger that initiates synthesis of the activator? Coupling translational activation with a morphological event occurring in the elongated spermatid is an exciting possibility to consider.

In elongating spermatids a specialized microtubule array called the manchette forms and may function in nuclear shaping.[46,47] It has been suggested this structure also plays a role in overall sperm differentiation by serving as a ‘track’ that is utilized for the movement of organelles, vesicles and mRNPs.[14,46,48] In the molecular screen that yielded *Prbp*, the *Spnr* gene (spermatid perinuclear RNA-binding protein), also was cloned and characterized.[14] *Spnr* encodes an RNA-binding protein that is expressed highly in elongating haploid germ cells, is localized to the manchette structure and appears to be a microtubule-associated protein (MAP).[49] Given that Spnr was cloned based on its ability to bind the *Prm-1* 3′ UTR, it may function as a MAP that links the *Prm-1* mRNA to the manchette and thereby plays a role in the putative subcellular localization of protamine mRNA molecules that are destined to be activated for translation at the nuclear periphery. Developmentally regulated protein production near the site of action has been established for a number of mRNAs, such as those involved in pattern formation during embryogenesis of *Drosophila*. In addition, there is correlative evidence for the involvement of the cytoskeleton in translational control processes (reviewed by Hesketh[50]). The protamine mRNAs might be targeted by such a regulatory mechanism.

Perspectives

Despite the prevalence of post-transcriptional regulation during spermatogenesis, relatively little is known about the mechanisms that control translational repression and activation of individual messages. Part of the reason for this stems from the lack of an *in vitro* system to study mammalian spermatogenesis. Development of such a system would greatly benefit the field and lead to significant advancements. Nonetheless, the availability of transgenesis and

gene-knockout technologies in the mouse does allow one to demonstrate function convincingly and the relative ease with which these can be performed, should permit the unambiguous assignment of function to individual genes. Numerous mRNAs have been shown to be under post-transcriptional control during spermatogenesis, yet in only one case, *Prm-1*, has it been demonstrated that sequences in the 3′ UTR control its translation. The *Prm-1* 3′ UTR appears to contain redundant translational repression elements and perhaps separate sequences required for translational activation. There is a need to study the regulation of other messages to determine if they contain similar or different *cis*-acting regulatory elements. To elucidate the mechanisms of translational regulation it is important to identify all of the factors involved. Despite significant progress in identifying protamine RNA-binding proteins, none of the proteins thus far described, PRBP,[41] TB-RBP,[23,24] or the 48/50 kDa Y box proteins,[39] have been shown to mediate the translational repression of the protamine mRNAs *in vivo*. Certain properties of these proteins, for example, the sequence-specific binding properties of the 48/50-kDa Y box proteins and TB-RBP, the ability of the binding site for the 48/50-kDa proteins to repress translation *in vivo* and the presence of the PRBP-binding site in a region of the *Prm-1* 3′ UTR shown to be sufficient for translational repression in transgenic mice, are desirable for putative translational repressors. But, these properties by themselves are not convincing. Proof that any of these genes is involved in translational control during spermatogenesis awaits genetic analysis of their function in the mouse.

Acknowledgements

I thank the members of my lab for careful review of this manuscript. Experiments in the Braun lab have been supported by grants from the March of Dimes Birth Defects Foundation and the National Institutes of Health (HD27215).

References

1. Olivieri G, Olivieri A (1965) Autoradiographic study of nucleic acid synthesis during spermatogenesis in *Drosophila melanogaster*. Mutation Res 2:366–380
2. Schmidt EE, Schibler U (1995) High accumulation of components of the RNA polymerase II transcription machinery in rodent spermatids. Development 121:2373–2383
3. Kierszenbaum AL, Tres IL (1975) Structural and transcriptional features of the mouse spermatid genome. J Cell Biol 65:258–270
4. Monesi V (1964) Ribonucleic acid synthesis during mitosis and meiosis in the mouse testis. J Cell Biol 22:521–532
5. Bellve AR (1979) The molecular biology of mammalian spermatogenesis. In: Finn CA, ed. Oxford Reviews of Reproductive Biology. London and New York: Oxford University Press, Vol 1, pp. 159–261
6. Balhorn R, Weston S, Thomas C, Wyrobek AJ (1984) DNA packaging in mouse spermatids. Synthesis of protamine variants and four transition proteins. Exp Cell Res 150:298–308
7. Kleene KC, Distel RJ, Hecht NB (1984) Translational regulation and deadenylation of a protamine mRNA during spermiogenesis in the mouse. Dev Biol 105:71–79
8. Mali P, Kaipia A, Kangasniemi M, Toppari J, Sandberg M, Hecht NB, Parvinen M (1989) Stage-specific expression of nucleoprotein mRNAs during rat and mouse spermiogenesis. Reprod Fertil Dev 1:369–382
9. Kleene KC (1996) Patterns of translational regulation in the mammalian testis. Mol Reprod Dev 43:268–281
10. Gold B, Fujimoto H, Kramer JM, Erickson RP, Hecht NB (1983) Haploid accumulation and translational control of phosphoglycerate kinase-2 messenger RNA during mouse spermatogenesis. Dev Biol 98:392–399
11. McCarrey JR, Berg WM, Paragioudakis SJ, Zhang PL, Dilworth DD, Arnold BL, Rossi JJ (1992) Differential transcription of *Pgk* genes during spermatogenesis in the mouse. Dev Biol 154:160–168
12. Robinson MO, Simon MI (1991) Determining transcript number using the polymerase chain reaction: *Pgk-2*, *mP2*, and *PGK-2* transgene mRNA levels during spermatogenesis. Nucleic Acids Res 19:1557–1562
13. Schumacher JM, Lee K, Edelhoff S, Braun RE (1995) Distribution of Tenr, an RNA binding protein, in a lattice-like network within the spermatid nucleus in the mouse. Biol Reprod 52:1274–1283
14. Schumacher JM, Lee K, Edelhoff S, Braun RE (1995) Spnr, a murine RNA-binding protein that is localized to cytoplasmic microtubules. J Cell Biol 129:1023–1032
15. Curtis D, Lehmann R, Zamore PD (1995) Translational regulation in development. Cell 81:171–178
16. Wickens M, Anderson P, Jackson RJ (1997) Life and death in the cytoplasm: messages from the 3′ end. Curr Opin Genet Dev 7:220–232
17. Braun RE, Peschon JJ, Behringer RR, Brinster RL, Palmiter RD (1989) Protamine 3′-untranslated sequences regulate temporal translational control and subcellular localization of growth hormone in spermatids of transgenic mice. Genes Dev 3:793–802
18. Lee K, Haugen HS, Clegg CH, Braun RE (1995) Premature translation of protamine 1 mRNA causes precocious nuclear condensation and arrests spermatid differentiation in mice. Proc Natl Acad Sci USA 92:12451–12455
19. Braun RE, Behringer RR, Peschon JJ, Brinster RL, Palmiter RD (1989) Genetically haploid spermatids are phenotypically diploid. Nature 337:373–376
20. Burgos MH, Fawcett DW (1955) Studies on the fine structure of the mammalian testis. I. Differentiation of the spermatids in the cat (*Felis domestica*). J Biophys Biochem Cytol 1:287–300
21. Fajardo MA, Haugen HS, Clegg CH, Braun RE (1997) Separate elements in the 3′ untranslated region of the mouse protamine 1 mRNA regulate translational repression and activation during murine spermatogenesis. Dev Biol (in press)
22. Braun RE (1990) Temporal translational regulation of the protamine 1 gene during mouse spermatogenesis. Enzyme 44:120–128
23. Kwon YK, Hecht NB (1991) Cytoplasmic protein binding to

highly conserved sequences in the 3′ untranslated region of mouse protamine 2 mRNA, a translationally regulated transcript of male germ cells. Proc Natl Acad Sci USA 88(9):3584–3588
24. Kwon YK, Hecht NB (1993) Binding of a phosphoprotein to the 3′ untranslated region of the mouse protamine 2 mRNA temporally represses its translation. Mol Cell Biol 13(10):6547–6557
25. Kuhn R, Schafer U, Schafer M (1988) *cis*-Acting regions sufficient for spermatocyte-specific transcriptional and spermatid-specific translational control of the *Drosophila melanogaster* gene *mst(3)gl-9*. EMBO 7:447–454
26. Schafer M, Kuhn R, Bosse F, Schafer U (1990) A conserved element in the leader mediates post-meiotic translation as well as cytoplasmic polyadenylation of a *Drosophila* spermatocyte mRNA. EMBO J 9:4519–4525
27. Schafer M, Nayernia K, Engel W, Schafer U (1995) Translational control in spermatogenesis. Dev Biol 172:344–352
28. Kempe E, Muhs B, Schafer M (1993) Gene regulation in *Drosophila* spermatogenesis: analysis of protein binding at the translational control element TCE. Dev Genet 14:449–459
29. Yanicostas C, Vincent A, Lepesant JA (1989) Transcriptional and post-transcriptional regulation contributes to the sex-regulated expression of two sequence-related genes at the *janus* locus of *Drosophila melanogaster*. Mol Cell Biol 9:2526–2535
30. Tafuri SR, Familari M, Wolffe AP (1993) A mouse Y box protein, MSY1, is associated with paternal mRNA in spermatocytes. J Biol Chem 268:12213–12220
31. Gu W, Kwon Y, Oko R, Hermo L, Hecht NB (1995) Poly (A) binding protein is bound to both stored and polysomal mRNAs in the mammalian testis. Mol Reprod Dev 40:273–285
32. Wolffe AP (1994) Structural and functional properties of the evolutionarily ancient Y-box family of nucleic acid binding proteins. Bioessays 16:245–251
33. Murray MT, Krohne G, Franke WW (1991) Different forms of soluble cytoplasmic mRNA binding proteins and particles in Xenopus laevis oocytes and embryos. J Cell Biol 112:1–11
34. Murray MT, Schiller DL, Franke WW (1992) Sequence analysis of cytoplasmic mRNA-binding proteins of Xenopus oocytes identifies a family of RNA-binding proteins. Proc Natl Acad Sci USA 89:11–15
35. Murray MT, Krohne G, Franke WW (1992) Different forms of soluble cytoplasmic mRNA binding proteins and particles in *Xenopus laevis* oocytes and embryos. J Cell Biol 112:1–11
36. Richter JD, Evers DC (1984) A monoclonal antibody to an oocyte-specific poly(A) RNA-binding protein. J Biol Chem 259:2190–2194
37. Tafuri SR, Wolffe AP (1990) *Xenopus* Y-box transcription factors: Molecular cloning, functional analysis, and developmental regulation. Proc Natl Acad Sci USA 87:9028–9032
38. Kwon YK, Murray MT, Hecht NB (1993) Proteins homologous to the *Xenopus* germ cell-specific RNA-binding proteins p54/p56 are temporally expressed in mouse male germ cells. Dev Biol 158:99–100
39. Fajardo MA, Butner KA, Lee K, Braun RE (1994) Germ cell-specific proteins interact with the 3′ untranslated regions of *Prm-1* and *Prm-2* mRNA. Dev Biol 166(2):643–653
40. Bouvet P, Matsumoto K, Wolffe AP (1995) Sequence-specific RNA recognition by the *Xenopus* Y-box proteins. An essential role for the cold shock domain. J Biol Chem 270:28297–28303
41. Lee K, Fajardo MA, Braun RE (1996) A testis cytoplasmic RNA-binding protein that has the properties of a translational repressor. Mol Cell Biol 16:3023–3034
42. Green SR, Mathews MB (1992) Two RNA-binding motifs in the double-stranded RNA-activated protein kinase, DAI. Genes Dev 6:2478–2490
43. St-Johnston D, Brown NH, Gall JG, Jantsch M (1992) A conserved double-stranded RNA-binding domain. Proc Natl Acad Sci USA 89:10979–10983
44. Wu XQ, Gu W, Meng X, Hecht NB (1997) The RNA-binding protein, TB-RBP, is the mouse homologue of translin, a recombination protein associated with chromosomal translocations. Proc Natl Acad Sci USA 94:5640–5645
45. Aoki K, Suzuki K, Sugano T, Tasaka T, Nakahara K, Kuge O, Omori A, Kasmi M (1995) A novel gene, *Translin*, encodes a recombination hotspot binding protein associated with chromosomal translocations. Nat Genet 10:167–174
46. Fawcett DW, Anderson WA, Phillips DM (1971) Morphogenetic factors influencing the shape of the sperm head. Dev Biol 26:220–251
47. Russell LD, Russell JA, MacGregor GR, Meistrich ML (1991) Linkage of manchette microtubules to the nuclear envelope and observations of the role of the manchette in nuclear shaping during spermiogenesis in rodents. Am J Anat 192(2):97–120
48. MacKinnon EA, Abraham JP (1972) The manchette in stage 14 rat spermatids: a possible structural relationship with the redundant nuclear envelope. Z Zellforsch Mikrosk Anat 124:1–11
49. Schumacher JM, Artzt K, Braun RE (1998) The spermatid perinuclear RNA binding protein SPNR, binds microtubules in vitro and associates with abnormal manchettes in vivo. Biol Reprod (in press)
50. Hesketh J (1994) Translation and the cytoskeleton: a mechanism for targeted protein synthesis. Mol Biol Rep 19(3):233–243