The poly(A)-binding protein partner Paip2a controls translation during late spermiogenesis in mice

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Introduction

Spermiogenesis is the process by which postmeiotic round spermatids (RSs) differentiate into elongated spermatids. The final step of spermiogenesis consists of dynamic morphological changes such as nuclear condensation, acrosome and sperm tail formation, and reorganization of organelles including the formation of the mitochondrial sheath along the midpiece of the flagellum (1). Regulated protein expression of components in each step is essential for proper germ cell differentiation.

While regulated gene expression is essential for germ cell development, transcription ceases at mid-spermiogenesis in RSs (2, 3). Gene expression in late spermiogenesis, therefore, is under translational control. mRNAs encoding proteins that are needed for late spermiogenesis are expressed in early spermatids and stored as translationally inactive messenger ribonucleoprotein particles (mRNPs). The translation of these mRNAs is associated with shortening of their poly(A) tail in late spermiogenesis. Poly(A)-binding protein (Pabp) plays an important role in mRNA stabilization and translation. Three Pabp-interacting proteins, Paip1, Paip2a, and Paip2b, have been described. Paip2a is expressed in late spermatids. To investigate the role of Paip2 in spermiogenesis, we generated mice with knockout of either Paip2a or Paip2b and double-KO (DKO) mice lacking both Paip2a and Paip2b. Paip2a-KO and Paip2a/Paip2b-DKO mice exhibited male infertility. Translation of several mRNAs encoding proteins essential to male germ cell development was inhibited in late spermiogenesis in Paip2a/Paip2b-DKO mice, resulting in defective elongated spermatids. Inhibition of translation in Paip2a/Paip2b-DKO mice was caused by aberrant increased expression of Pabp, which impaired the interaction between eukaryotic initiation factor 4E (eIF4E) and the cap structure at the 5' end of the mRNA. We therefore propose a model whereby efficient mRNA translation in late spermiogenesis occurs at an optimal concentration of Pabp, a condition not fulfilled in Paip2a/Paip2b-DKO mice.

Polyadenylation and translational control via the poly(A) tail play important roles during late spermiogenesis. For instance, mRNAs encoding basic nuclear proteins such as protamines (Prms) and transition proteins (Tps) are produced in RSs and stored as mRNPs, which become translationally activated in elongated spermatids (10–16). Prm and Tp mRNAs that are translationally repressed in RSs contain poly(A) tails of approximately 180 nucleotides. They become activated in ESs, concomitant with the shortening of the poly(A) tail to approximately 30 nucleotides (17). The mechanism of translational activation and what triggers the shortening of the poly(A) tails are not understood (9).

RNA-binding proteins function in the stabilization and translation of mRNAs in differentiating male germ cells (18). For example, the germ cell Y-box protein Msy2 is a DNA/RNA-binding protein that acts as a stabilizer and a translational suppressor of mRNAs in RNP complexes (19, 20). Msy2-KO mice display spermatogenic arrest (20, 21). Another RNA-binding protein, polyuridylic acid tract binding protein 2 (Ptbp2), stabilizes the testis-specific glycolytic enzyme Pgk2 mRNA and delays its translation for up to 2 weeks (22). The poly(A)-binding protein (Pabp) contains 4 RNA recognition motifs (RRMs) and a proline-rich C-terminal domain and binds to the poly(A) tail of the mRNA with a periodicity of about 25–27 nucleotides (23, 24). Pabp functions in mRNA biogenesis, mRNA stabilization, and translation (25–27). In spermatogenesis, Pabp is associated with RNPs and polysomal mRNAs, suggesting that Pabp plays a role in both mRNA storage and translation (28). There are two isoforms of Pabp in mouse spermatogenic cells: Pabpc1 and the testis-specific Pabpc2 (29). Both isoforms are present in pachytene spermatocytes (PSs) and RSs, while only Pabpc1 is expressed in elongating spermatids (30). Interestingly, whereas Pabpc1 is associated with both monosomes and polysomes, Pabpc2 is present only in monosomes, suggesting that the isoforms serve distinct functions during spermatogenesis (30).

Pabp activity is modulated in metazoans by two Pabp-interacting proteins (Paips): Paip1 and Paip2 (of which there are two related gene products in mammals, Paip2a and Paip2b). Paip1 stimulates translation through its interaction with eukaryotic ribonucleoprotein particles (mRNPs). The translation of these mRNAs is subsequently associated with shortening of their poly(A) tails in late spermiogenesis. Poly(A)-binding protein (Pabp) plays an important role in mRNA stabilization and translation. Three Pabp-interacting proteins, Paip1, Paip2a, and Paip2b, have been described. Paip2a is expressed in late spermatids. To investigate the role of Paip2 in spermiogenesis, we generated mice with knockout of either Paip2a or Paip2b and double-KO (DKO) mice lacking both Paip2a and Paip2b. Paip2a-KO and Paip2a/Paip2b-DKO mice exhibited male infertility. Translation of several mRNAs encoding proteins essential to male germ cell development was inhibited in late spermiogenesis in Paip2a/Paip2b-DKO mice, resulting in defective elongated spermatids. Inhibition of translation in Paip2a/Paip2b-DKO mice was caused by aberrant increased expression of Pabp, which impaired the interaction between eukaryotic initiation factor 4E (eIF4E) and the cap structure at the 5' end of the mRNA. We therefore propose a model whereby efficient mRNA translation in late spermiogenesis occurs at an optimal concentration of Pabp, a condition not fulfilled in Paip2a/Paip2b-DKO mice.

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initiation factor 3 (eIF3) (31). Paip2 is a translational inhibitor, as it competes with eIF4G for Pabp binding and decreases the affinity of Pabp for the poly(A) tail (32, 33). Paip2 is a highly acidic protein that contains two separate Pabp-binding motifs (PAMs) (34). The two isoforms of Paip2 bind to Pabp but are expressed differentially in tissues, implying tissue-specific functions and responses to distinct stimuli (35).

Given Pabp's important role in spermatogenesis (36–38), we reasoned that Paip2 may play a key role in this process. To investigate the physiological role of Paip2 in translational control during spermatogenesis, Paip2a-KO, Paip2b-KO, and Paip2a/Paip2b-double-KO (Paip2a/Paip2b-DKO) mice were generated. Here, we describe the phenotype of these mice with respect to fertility and propose a model for translational control in late spermiogenesis via the control of Pabpc1-Paip2a association. Our data implicate Paip2a as an important player in translational control of late spermiogenesis.

**Results**

**Mouse tissue distribution of Paip2a and Paip2b.** Paip2a and Paip2b expression was assessed in tissues by Western blot analysis. Paip2a protein was highly expressed in pancreas and testis, while Paip2b was highly expressed in pancreas, but barely in testis (Figure 1A). Paip2a was also expressed in brain, where the expression of Paip2b was minimal. Paip2a and Paip2b were barely detected in liver. Pabp was expressed at high levels in pancreas and testis and at lower levels in brain and liver. Thus, expression of the Paip2 isoforms and Pabp is tissue specific, with overlapping high expression of Paip2a and Pabp in testis. Paip2a and Paip2b expression in other reproductive tissues was assessed by Western blot analysis (Figure 1B). Paip2a was weakly expressed in male reproductive tissues such as epididymis, seminal vesicle, and prostate. However, Paip2a was highly expressed in female reproductive tissues such as ovary, uterus, and vagina. Paip2b was weakly expressed in testis but barely expressed in other male or female reproductive tissues. Pabp also showed high expression levels in testis, but not in other reproductive tissues.

To determine which testicular cells express Paip2a, we performed immunohistochemistry with an affinity-purified Paip2 antibody (Figure 1C). This antibody was generated against full-length human PAIP2A and weakly cross-reacts with PAIP2B (35). Paip2a was expressed only in germ cell cytoplasm and started being expressed in spermatids from steps 7 to 8 until the end of spermiogenesis, indicating that it may function during the last steps of this process.

**Generation of Paip2a-KO, Paip2b-KO, and Paip2a/Paip2b-DKO mice.** To study the role played by Paip2a in late spermiogenesis, we generated knockout mice. A Paip2a targeting vector was electroporated into R1 ES cells derived from male blastocysts of a hybrid of two 129/SvJ substrains. After G418 selection, the LoxP-flanked Neo cassette was removed by transient expression of Cre recombinase (Figure 2A). The targeted ES cell clones were injected into C57BL/6 blastocysts, and subsequent chimeric male mice were mated with

**Table 1**

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Male WT, Paip2a-KO, Paip2b-KO, and Paip2a/Paip2b-DKO mice (10–12 weeks old) were bred with WT females for 1 year. The number of pups per litter is shown. Values are presented as mean ± SEM of 10 mice.
C57BL/6J females to achieve Paip2a allele germline transmission. The second and third exons were removed to generate Paip2a-KO mice by crossing with the CMV-Cre transgenic mouse. Paip2a heterozygotes were crossed to generate Paip2a-KO mice as confirmed by Southern blot analysis (Figure 2C and Supplemental Figure 1, A and C; supplemental material available online with this article; doi:10.1172/JCI43350DS1). Paip2b-KO mice were also generated (Figure 2, B and C, and Supplemental Figure 1, B and D) and crossed with Paip2a-KO mice to obtain Paip2a/Paip2b-DKO mice. The genotypes were confirmed by PCR targeting the loxP sites (Figure 2D). Western blot analysis confirmed the absence of Paip2a and Paip2b in testes (Figure 2E) and epididymides (Figure 2F).

Body weight of Paip2a/Paip2b-DKO mice was lower than that of WT mice (5.6% less than WT, P < 0.05 using an unpaired t test) (Figure 3A). Statistically significant differences in weight between Paip2a/Paip2b-DKO and WT mice were observed for epididymis (19.8% lower than WT) and for prostate (71.2% lower than WT) (Figure 3B). There was no significant difference in weight of testis and seminal vesicle (non-normalized organ weights are shown in Supplemental Figure 2).

Spermatogenesis was monitored by counting the number of sperm heads in testes and epididymides (Figure 3C). While no difference was observed in testis weight between Paip2a/Paip2b-DKO and WT strains, a significant decrease (64% ± 13%) in the number of sperm heads was observed in Paip2a/Paip2b-DKO testes compared to WT, indicating a complete lack of sperm production in these mice.

The lack of Paip2a resulted in male infertility, with no litters in Paip2a-KO or Paip2a/Paip2b-DKO males. Mating of transgenic females with WT males was also performed. No change in the litter per female or in the size of each litter was observed (data not shown), thus demonstrating that even though Paip2a is expressed in female reproductive organs, it is not essential for female fertility. Given that Paip2a-KO and Paip2a/Paip2b-DKO mice exhibit the same infertile phenotype, we pursued our studies with Paip2a/Paip2b-DKO mice because of the possibility, albeit remote, that the Paip2 antibody might cross-react with Paip2b in immunohistochemical assay at low intensity.

Figure 2
Generation of Paip2a/Paip2b-KO mouse. (A) Targeted disruption of Paip2a. Exons 2, 3, and 4 are depicted as filled boxes. The targeting regions are shown as bold lines. Filled arrows indicate 5′ probe and 3′ probe for Southern blot analysis. Open arrows indicate loxP sites. Gray arrows indicate primers for genotyping (P1, P2, P3, and P4). (B) Targeted disruption of Paip2b. Exons 3 and 4 are shown as filled boxes. Gray arrows indicate primers for genotyping (P5, P6, P7, and P8). (C) Southern blot analysis of WT, Paip2a heterozygote, Paip2a-KO, Paip2b heterozygote, and Paip2b-KO mice probed by 5′ probes (see also Supplemental Figure 1). (D) Genotyping of WT, Paip2a-KO, Paip2b-KO, and Paip2a/Paip2b-DKO mice. Primer sets of P1 and P2, P3 and P4, and P1 and P4 were used to detect the first and second loxP sites and the recombinant loxP site of Paip2a, respectively. Primer sets of P5 and P6, P7 and P8, and P5 and P8 were used to detect the first loxP site, the second loxP site, and the recombinant loxP site of Paip2b, respectively. (E) Western blot analysis of Paip2a, Paip2b, and Pabp in testes. (F) Western blot analysis of Paip2a, Paip2b, and Pabp in epididymides.
of sperm heads was detected in testes of Paip2a/Paip2b-DKO mice as compared with those of WT mice. This was consistent with a dramatic decrease in the number of sperm heads in caput/corpus epididymis (99.2% ± 0.4%), as well as cauda epididymis (99.8% ± 0.2%), in which some animals displayed a total absence of spermatozoa (Figure 3C).

To resolve the apparent differences between changes in weight and sperm counts, we undertook histological examination of testes and epididymides of WT and Paip2a/Paip2b-DKO mice. All spermatogenic stages could be observed in Paip2a/Paip2b-DKO testes, with normal gross morphological structures of Sertoli cells, spermatogonia, spermatocytes, and elongating spermatids (data not shown). However, aberrant spermatozoa were observed in the epithelia along the lumen of seminiferous tubules at stage VII in testes of Paip2a/Paip2b-DKO mice, which were not released into the lumen, as in WT mice (Figure 3D, arrowheads in Paip2a/Paip2b-DKO). The retained elongated spermatids were present even at stages IX–XII in testes of Paip2a/Paip2b-DKO mice (arrowheads in Figure 3D, Supplemental Figure 3B, and Supplemental Figure 4D). Strikingly, two populations of spermatids that are composed of a normal population of elongating spermatids (arrows in Figure 3D) and a population of aberrant condensed spermatids (arrowheads in Figure 3D) were observed through stages IX–XII in Paip2a/Paip2b-DKO mice. In contrast, spermatids in WT mice began elongating and formed a homogeneous population of elongating spermatids during stages IX–XII (arrows in Figure 3D), suggesting that the spermiation process, i.e., the release of completed spermatozoa into the lumen of seminiferous tubules, is impaired in Paip2a/Paip2b-DKO mice. Significantly, spermatids exhibiting aberrant morphology in Paip2a/Paip2b-DKO testis corresponded to those expressing Paip2a (Figure 1C) in late spermiogenesis. These observations demonstrate an important role of Paip2a in germ cell differentiation during late spermiogenesis.

Few sperm heads were observed in the lumen of caput and cauda epididymides of Paip2a/Paip2b-DKO mice as compared with WT mice (Figure 3E). Aberrant round structures were apparent in cauda epididymis of Paip2a/Paip2b-DKO mice (Figure 3E and Supplemental Figure 4D). The same phenotype was seen in tes-
Abnormal Pabp expression in late spermiogenesis in Paip2a/Paip2b-DKO mice. (A and B) Immunohistochemistry of Pabp in testes of WT (A) and Paip2a/Paip2b-DKO (B) mice. Stages I–IV, V, VII, and X–XII are shown. Arrows show aberrant Pabp expression in elongated spermatids at stages V and VII of Paip2a/Paip2b-DKO mice. An asterisk indicates a layer of aberrant elongated spermatids in which Pabp is slightly expressed at stages X–XII in Paip2a/Paip2b-DKO testis. Scale bars: 25 μm. (C and D) Immunohistochemistry of Pabp in caput and cauda epididymides of WT (C) and Paip2a/Paip2b-DKO (D) mice. Scale bars: 50 μm.

Abnormal Pabp expression in late spermiogenesis in Paip2a/Paip2b-DKO mice. To investigate the molecular basis of the Paip2a/KO phenotype, we performed immunohistochemistry to determine Pabp expression in testes of WT and Paip2a/Paip2b-DKO mice. In WT mice, Pabp was first expressed in the cytoplasm of PSs, reached its peak expression in step 7 and 8 of spermiogenesis, diminished as spermatids underwent nuclear condensation, and disappeared around step 15 of spermiogenesis (Figure 4A) (28). In sharp contrast, Pabp was still expressed in late spermiogenesis in Paip2a/Paip2b-DKO mice (Figure 4B): at stages X–XII, a layer of aberrant elongated spermatids was observed in which Pabp was still being expressed (asterisk in Figure 4B). Moreover, while Pabp was absent in the lumen of cauda epididymides in WT mice (Figure 4C), it was detected in Paip2a/Paip2b-DKO mice (Figure 4D). These data clearly indicate that Paip2a modulates Pabp expression and raise the possibility that translational control through Pabp-Paip2a interaction is important at the final steps of spermiogenesis.

Impairment of formation of mitochondrial sheath and chromatin condensation in Paip2a/Paip2b-DKO mice. To determine how the defects in elongated spermatids of Paip2a/Paip2b-DKO mice affect ultrastructure and epididymides of Paip2a-KO mice (Supplemental Figure 4B) but not in Paip2b-KO mice (Supplemental Figure 4C). These data demonstrate that male infertility is caused, at least in part, by impaired release of spermatozoa from seminiferous tubules and migration from testis to epididymis.

Anomalous Pabp expression in late spermiogenesis in Paip2a/Paip2b-DKO mice. To understand the molecular basis of the loss of Paip2a, further supporting a model whereby Paip2a plays an important regulatory role in late spermiogenesis. Consistent with the existence of abnormal spermatids in testis, abnormal spermatogenesis were also present in cauda epididymides of Paip2a/Paip2b-DKO mice (Figure 5J–L) as compared with WT mice (Figure 5J–L). vacation of various size in the majority of nuclei were detected at stage VII of spermatogenesis in Paip2a/Paip2b-DKO mice, while these structures were rare in WT spermatids (Figure 5C and F). The large number of cellular defects observed in Paip2a/Paip2b-DKO mice suggests that key components needed for cell differentiation in late spermiogenesis are not generated properly as a consequence of the loss of Paip2a.
in \textit{Prm1}, \textit{Tp1}, and \textit{Tp2} mRNA levels of \textit{Paip2a/Paip2b}-DKO testes as compared with WT testes as determined by real-time quantita-
tive PCR (Supplemental Figure 5). These data indicate that \textit{Paip2a} is required for efficient synthesis of \textit{Prm1}, \textit{Tp1}, and \textit{Tp2} proteins. \textit{Tp2} protein expression as assessed by immunohistochemistry was also reduced in testes of \textit{Paip2a/Paip2b}-DKO mice (arrowheads) are shown. (G–L) Spermatozoa in cauda epididy-
mites from WT (G–I) and \textit{Paip2a/Paip2b}-DKO (J–L) mice. Mitochondria (arrows), ectopic residual bodies (arrowheads), and bubble-like structures in nucleus (asterisk) are shown. Original
magnification, A and D: \times8100, B and E: \times10,200, C and F: \times24,400, G and J: \times2,120, H and K: \times13,800, I and L: \times24,400.

To investigate in which germ cells \textit{Paip2a}, \textit{Paip2b}, \textit{Pabpc1}, and \textit{Pabpc2} are expressed, we analyzed PSs, RSs, and ESs from WT and \textit{Paip2a/Paip2b}-DKO mice by Western blot analy-
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for poly(A) shortening of mRNAs encoding proteins required for germ cell differentiation in late spermiogenesis is different in WT and Paip2a/Paip2b-DKO mice. The possible explanation for this difference is addressed in the Discussion.

Excess Pabp inhibits translation in late spermiogenesis. It is possible that translation is impaired by the increased Pabpc1 expression in late spermiogenesis of Paip2a/Paip2b-DKO mice. Pabpc1 stimulates translation, but the effect of excess Pabp on translation is not known. To address this question, we used established cell-free translation extracts because it is not possible to prepare a translationally competent cell-free extract from testis, primarily due to limited material. Pabp was depleted from a Krebs-2 ascites cell extract in which translation was inhibited relative to the Pabp-depleted cell extract (compare bars 7–10). At this concentration of Pabp, translation was even inhibited relative to the Pabp-depleted cell extract (compare bars 10 and 2). Thus, these results lead to a prediction that an increase in the abundance and activity of Pabp in elongated spermatids of Paip2a/Paip2b-DKO mice would cause translation inhibition.

To investigate whether Paip2a can reverse the translation inhibition caused by excess Pabp, we added recombinant GST-tagged PAIP2A to a Pabp-depleted Krebs-2 ascites cell extract in which translation was inhibited by PABP (30 μg/ml) (Figure 7B). Increasing PAIP2A concentrations restored translation (Figure 7B, bars 2–7). Thus, Paip2a is expected to function in a stimulatory manner under conditions of Pabp abundance, such as those in late spermiogenesis.

At the optimal concentration, Pabp largely exerts its stimulatory activity on translation through binding to eIF4G, which is a subunit of the eIF4F heterotrimeric complex eIF4E/4G/4A, and stabilizing the association of eIF4E with the cap structure (40). To investigate whether excess Pabp inhibits the activity of eIF4E, we analyzed the interaction of eIF4E with the cap structure by chemical crosslinking in mock- and Pabp-depleted rabbit reticulocyte lysate (RRL) using Luc mRNA 32P-labeled at the 5’ cap. We previously established this assay as a reliable readout of eIF4E activity (41). eIF4E and eIF4A interacted specifically with the cap structure (Figure 7C, lane 1), as this interaction was inhibited by the cap analog m’GDP (9% of control; lane 2). In the Pabp-depleted RRL, eIF4E crosslinking was impaired (26% of control; lane 3). The addition of PABP (15 μg/ml) to Pabp-depleted RRL largely restored eIF4E crosslinking to the cap structure (79% of control; lane 4). Of significance, excess PABP (30 μg/ml) dramatically reduced eIF4E

![Figure 6](http://www.jci.org) Translation inhibition in late spermiogenesis of Paip2a/Paip2b-DKO mice. (A) Levels of basic nuclear proteins (Prm1, Tp1, Tp2, and histone H1) from testes of WT and Paip2a/Paip2b-DKO mice were analyzed by acid-urea polyacrylamide gel electrophoresis. (B) Tp2 expression in testes at stages X–XII from WT and Paip2a/Paip2b-DKO mice analyzed by immunohistochemistry. Arrows indicate Tp2-positive spermatids, and arrowheads indicate Tp2-negative spermatids. Scale bar: 10 μm. (C) Expression of Paip2a, Paip2b, Pabp1, and Pabpc2 in PSs, RSs, and ESs of WT (lanes 1–3) and Paip2a/Paip2b-DKO (lanes 4–6) mice analyzed by Western blotting. (D) Prm1 mRNA in testes from WT, Paip2a-KO, Paip2b-KO, and Paip2a/Paip2b-DKO mice analyzed by Northern blot analysis. Numbers on the right denote nucleotides, and arrows indicate ladder RNA fragments. (E) Ladder RNA fragments were generated by cleavage of poly(A) tail. Total testis RNA of WT and Paip2a/Paip2b-DKO mice was incubated without (−) or with (+) oligo(dT) and then treated with RNase H, followed by Northern blot analysis. Ribosomal 18S RNA (1,870 nucleotides) was used as a control for the absence of nonspecific RNA degradation during this experiment.

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crosslinking (2% of control; lane 5). Thus, excess PABP impairs the interaction between eIF4E and the cap structure, resulting in translation inhibition. Nonspecific binding of PABP to the mRNA was observed (lanes 4 and 5), as this interaction was not inhibited by m7GDP (Supplemental Figure 7B). Although these results were obtained in a system different from testis, our findings are consistent with a model whereby Paip2a promotes efficient translation by inhibiting Pabp in late spermiogenesis (Figure 7D).

**Discussion**

In the present study, we demonstrated that Paip2a is required for late spermiogenesis through maintenance of Pabp homeostasis. In Paip2a/ Paip2b-DKO mice, the translation of mRNAs encoding proteins such as basic nuclear proteins needed for late spermiogenesis was dramatically reduced, which was associated with persistent expression and elevated activity of Pabp. We propose a model whereby efficient mRNA translation in late spermiogenesis
occurs at an optimal concentration of active Pabp, a condition not fulfilled in Paip2a/Paip2b-DKO mice. This model posits that in the absence of Paip2a buffering, excess Pabp competes with eIF4G for mRNAs binding, resulting in reduced association of eIF4E with the cap structure and translational inhibition (42). While not prominently considered by current translation models (43), Pabp binding to internal mRNAs sites has been documented in several studies (44–46). The 4 RRM of Pabp have apparently different functions. While the combination of RRM domains 1 and 2 binds oligo(A) with the same affinity as intact Pabp, that of RRM domains 3 and 4 recognizes oligo(A) with reduced affinity and specificity (47, 48). Thus, Pabp interaction with the mRNA body is likely due to the promiscuous binding of RRM domains 3 and 4. In binding to the 5′ UTR, Pabp would compete with initiation factors for translation, i.e., display an activity characteristic of general RNA-binding proteins (41). In support of this idea, we showed that high concentrations of Pabp inhibit translation in vitro and also decrease the assembly of the eIF4E complex on the mRNA structure, as measured by eIF4E crosslinking. This is in sharp contrast to the prominent enhancement of translation that Pabp displays at sub-saturating concentrations (40). In addition, free Pabp can potentially compete with poly(A)-bound Pabp to disrupt mRNA circularization and translation, i.e., display an activity characteristic of general RNA-binding proteins (41, 42). While the combination of RRM domains 1 and 2 binds oligo(A) strongly and efficiently (43), Pabp binding to internal mRNA sites has been documented in several studies (44–46). The 4 RMs of Pabp have apparently different functions. While the combination of RRM domains 1 and 2 binds oligo(A) with the same affinity as intact Pabp, that of RRM domains 3 and 4 recognizes oligo(A) with reduced affinity and specificity (47, 48). Thus, Pabp interaction with the mRNA body is likely due to the promiscuous binding of RRM domains 3 and 4. In binding to the 5′ UTR, Pabp would compete with initiation factors for translation, i.e., display an activity characteristic of general RNA-binding proteins (41). In support of this idea, we showed that high concentrations of Pabp inhibit translation in vitro and also decrease the assembly of the eIF4E complex on the mRNA structure, as measured by eIF4E crosslinking. This is in sharp contrast to the prominent enhancement of translation that Pabp displays at sub-saturating concentrations (40). In addition, free Pabp can potentially compete with poly(A)-bound Pabp to disrupt mRNA circularization and translation, i.e., display an activity characteristic of general RNA-binding proteins (41). This feature allows Pabp to play a major role in spermatozoa release (50). Studying the process of spermiation is not well understood (1), but it involves interactions between Sertoli and germ cells (49). Sertoli cells play a major role in spermatozoa release (50). Studying the mechanism of spermatogenesis is essential for understanding how defects in spermatogenesis cause idiopathic male infertility (51). Two knockout mouse models have been reported to exhibit a spermatogenesis defect. Null mice for the transcription factor HMG box 8 (Sox8) show age-dependant spermatogenesis disorganization and spermatogenesis defect (52); and cleavage stimulation factor, 3′ pre-RNA subunit 2, tau-null (Csf2r-null) mice have a failure of spermatogenesis (53). Spermatogonia in testes and cauda epididymides of Paip2a/Paip2b-DKO mice exhibit multiple morphological abnormalities, including impairment of Flagellum formation associated with the absence of the mitochondrial sheath in the middle piece. We have also described a failure of Sertoli cells to remove residual bodies, a detachment of the acrosome from the nucleus, and aberrant vacuoles in the chromatin suggesting an impairment of the chromatin condensation process. These defects may be explained by Paip2a’s translational control of mRNAs encoding proteins needed for these processes. Paip2a expression is altered in late spermiogenesis of Paip2a/Paip2b-DKO mice. Paip2a protein levels are reduced in PABP-depleted HeLa cells due to its ubiquitination and degradation (54). Thus, Pabp and Paip2a affect each other’s cellular abundance. One possible explanation among many for the aberrant Pabp expression in Paip2a/Paip2b-DKO mice is that mRNAs encoding components responsible for Pabp degradation cannot be translated in late spermiogenesis because of the lack of Paip2a.

In summary, we have demonstrated a novel role for Paip2a in male germ cell development during late spermiogenesis. Considering the morphological abnormalities observed in elongated spermatids of Paip2a-KO and Paip2a/Paip2b-DKO mice, it is conceivable that translational control by Paip2a-Pabp interaction affects the synthesis of key proteins essential for male germ cell maturation during late spermiogenesis. Further studies on the Paip2a mouse model could provide important information about male germ cell development and fertility.

Methods

**Targeted disruption of Paip2a.** A PCR fragment amplified with a primer set of Paip2a 5′ Fwd and Rev (Supplemental Table 1) was used as a probe to isolate genomic BAC DNA clone 103F10 from the 129/Sv mouse BAC genomic library RPCI-22. The targeting vector was constructed by recombination (55), and routine cloning methods were employed with a 10.5-kbp mouse Paip2a genomic fragment from clone 103F10, as illustrated in Figure 2A. The final targeting fragment was excised from its cloning vector backbone by NotI and electrooporated into R1 ES cells. Southern blot analysis was performed with two probes corresponding to the 5′ and 3′ sequences outside the targeting region, as indicated in Figure 2A. To generate the 5′ and 3′ probes, the primer sets (Paip2a 5′ Fwd and Rev for the 5′ probe and Paip2a 3′ Fwd and Rev for the 3′ probe) listed in Supplemental Table 1 were used. After the LoxP-flanked Neo cassette was eliminated by subsequent transient transfection with a cre recombinase expression vector, these genomic engineering steps in ES cells resulted in two non-insertions: the first 100-bp LoxP-containing sequence at the BstBI site is located 1 kb upstream of exon 2, and the second 107-bp LoxP-containing sequence at the BlnI site is located 302 bp downstream of exon 3 (Supplemental Table 2). Chimeric male mice were generated by the injection of the Paip2a-targeted ES cells into C57BL/6 blastocysts. The resultant chimeric mice were crossed with C57BL/6J mice for the Paip2a allele germline transmission to obtain Paip2afl/fl mice. After germline transmission, Paip2aflox/fl mice were crossed with CMV-Cre transgenic mice constitutively expressing a cre gene under the control of the CMV promoter (56) to eliminate the second and third exons of Paip2a. Mice were then backcrossed with C57BL/6J mice from The Jackson Laboratory for 10 generations using JAX Speed Congenic Service. The mice were genotyped with the primer sets for amplification of the first LoxP site flanking region (Paip2a P1 and P2; 369 bp), the second LoxP site flanking region (Paip2a P3 and P4; 1,183 bp), and the recombinant loxP site flanking region (Paip2a P1 and P4; 660 bp) (Figure 2, A and D, and Supplemental Table 1). Ten- to 12-week-old mice were used for experiments. All experiments were approved by the McGill University Animal Care Committee.

**Targeted disruption of Paip2b.** To generate Paip2b-KO mice, PCR fragment amplified with a primer set of Paip2b 5′ Fwd and Rev (Supplemental Table 3) was used as a probe to isolate genomic BAC DNA clone 249C9 from the 129/Sv mouse BAC genomic library RPCI-22. The targeting vector was constructed using a 13-kbp mouse Paip2b genomic fragment from clone 249C9 as illustrated in Figure 2B. The final targeting fragment was excised from its cloning vector backbone by NotI and electrooporated into R1 ES cells. The LoxP-flanked Neo cassette was eliminated. Southern blot analysis was performed with two probes corresponding to the sequences outside the targeting region, as indicated in Figure 2B, with the primer sets (Paip2b 5′ Fwd and Rev for the 5′ probe and Paip2b 3′ Fwd and Rev for the 3′ probe) listed in Supplemental Table 3. These genomic engineering steps in ES cells resulted in two insertions: a 115-bp LoxP-containing area.
sequence at the first SpeI site located 1,053 bp upstream of exon 3, and a 112-bp LoxP-containing sequence at the second SpeI site located 529 bp downstream of exon 3 (Supplemental Table 4). {Paip2b-KO mice in which the third exon was removed were generated as described for the targeted disruption of Paip2a. The mice were genotyped with the primer sets for amplification of the first LoxP site flanking region (Paip2b P5 and P6; 339 bp), the second LoxP site flanking region (Paip2b P7 and P8; 546 bp), and the recombined LoxP site flanking region (Paip2b P5 and P8; 449 bp) (Figure 2, B and D, and Supplemental Table 3).

Generation of Paip2a/Paip2b-DKO mice. Paip2a-KO and Paip2b-KO heterozygous mice were intercrossed to obtain Paip2a/Paip2b-DKO mice.

Antibodies. Anti-Paip2 antibody (P0087) was purchased from Sigma-Aldrich and used as Paip2a-specific antibody for Western blot analysis. Paip2b antibody (35) was used as Paip2b-specific antibody for Western blot analysis. Paip2 antibody (32) was purified with AminoLink Plus Immobilization Kit (Pierce) according to the manufacturer’s instructions and used for immunohistochemistry. This antibody mainly reacts with Paip2a and weakly cross-reacts with Paip2b. PAPBI antibody (no. 4992) was purchased from Cell Signaling Technology and used for Western blot analysis and immunohistochemistry. Antibodies against Pabp, Pabpc1, and Pabpc2 were a gift from T. Baba (University of Tsukuba, Tsukuba, Ibaraki, Japan) (30) and used for Western blot analysis. Monoclonal mouse anti-human smooth muscle actin clone IA4 (N0851) was purchased from Dako to detect actin, and monoclonal anti-β-actin antibody (A5441) was purchased from Sigma-Aldrich for Western blot analysis. Histone H1 (AE-4) antibody (sc-8030) was purchased from Santa Cruz Biotechnology Inc. for immunohistochemistry. This antibody mainly reacts with Paip2a and weakly cross-reacts with Paip2b. PABP1 antibody (no. 4992) was purchased from Santa Cruz Biotechnology Inc. and used for immunohistochemistry. Paip2, Pabp, and Tp2 were detected with DAB Peroxidase Substrate Kit (Vector Laboratories) according to the manufacturer’s instructions and then counterstained with hematoxylin. Pictures were taken using a CoolSnap camera attached to a Leica DM LB2 microscope.

Cell separation. Spermatogenic cells were obtained by cell separation as described previously (60). Briefly, the testes of 6 mice were decapsulated and digested by enzymatic treatment at 34°C with 0.5 mg/ml collagenase (Sigma-Aldrich, C9891) for 12 minutes, followed by 0.5 mg/ml trypsin (Sigma-Aldrich, T8003) for 16 minutes. The cell suspension was filtered through a nylon mesh, and washed with RPMI medium containing 3% FBS and 1% penicillin/streptomycin. The pellets were collected and re-suspended in cold water containing protease inhibitor and incubated with 3% H2O2 for 10 minutes at room temperature. To detect Paip2, slides were boiled for 5 minutes with 0.01 M citrate buffer (pH 6.0) and incubated with blocking buffer (PBS containing 10% goat serum, 0.1% Triton X-100, and 5% BSA fraction V) for 30 minutes at room temperature. Slides were then incubated with blocking buffer containing affinity-purified Paip2 antibody (1:1,000), PABP1 antibody (1:1,000), or Tp2 antibody (1:1,000) overnight at 4°C. After washing in PBS, slides were incubated with biotinylated goat anti-rabbit IgG and subsequently with VECTASTAIN ABC reagents (Vector Laboratories) according to the manufacturer’s instructions. Paip2, Pabp, and Tp2 were detected with DAB Peroxidase Substrate Kit (Vector Laboratories) according to the manufacturer’s instructions and then counterstained with hematoxylin. Pictures were taken using an electron microscope (Philips 410 Electron Microscope).

Electron microscopy. To prepare samples for electron microscopy, testes and epididymides were fixed in formaldehyde and osmium tetroxide and post-fixed in 1% osmium tetroxide and 1.5% potassium ferrocyanide and embedded in epoxy resin, and then analyzed using an electron microscope (Philips 410 Electron Microscope).

Analysis of basic nuclear proteins. Total basic nuclear protein extracts were prepared as described previously (61, 62). Briefly, testes were homogenized in cold water containing protease inhibitor cocktail (10 µl/ml) (Sigma-Aldrich) and 0.1 mM PMSF. The cell suspension was filtered through 4 layers of gauze, and the filtrate was centrifuged at 600 g for 10 minutes. The pellet was suspended in cold water containing protease inhibitor and PMSF and sonicated for 1 minute, followed by incubation in water containing protease inhibitor and 10 mM DTT for 30 minutes. Basic nuclear proteins were extracted with 0.5 M HCl and precipitated overnight with 25% TCA. Resultant pellets were washed with acidified acetone, followed by aceton. Proteins (50 µg) were separated by electrophoresis in acid-urea 15% polyacrylamide gels. The proteins were blotted onto a Hybond polyvinylidene difluoride membrane in 0.7% acetic acid. Membranes were blocked in 5% nonfat milk in PBS containing 0.1% Tween 20 and probed with primary antibodies against Paip2a, Paip2b, Pabp, Pabpc1, and Pabpc2 at a dilution of 1:2,000 overnight at 4°C and subsequently with secondary antibody (ECL anti-rabbit IgG horseradish peroxidase-linked whole antibody [GE Healthcare]). Immunoreactive proteins were visualized using enhanced chemiluminescence (PerkinElmer).

Sperm count. The testis, the caput/corpus epididymis, and the cauda epididymides were sonicated twice for 15 seconds separated by a 30-second interval in 0.9% saline, 0.1% merthiolate, and 0.05% Triton X-100 using a sonicator (VWR International). Heads of spermatozoa were counted using a hemocytometer.

Immunohistochemistry. Testes were fixed in modified Davidson’s solution to detect Paip2 and Pabp (Electron Microscopy Sciences). Testes were fixed in Bouin solution for Tp2. Paraffin sections (5 µm) were rehydrated and incubated with 3% H2O2 for 10 minutes at room temperature. To detect Tp2, slides were boiled for 5 minutes with 0.01 M citrate buffer (pH 6.0) and incubated with blocking buffer (PBS containing 10% goat serum, 0.1% Triton X-100, and 5% BSA fraction V) for 30 minutes at room temperature. Slides were then incubated with blocking buffer containing affinity-purified Paip2 antibody (1:1,000), PABP1 antibody (1:1,000), or Tp2 antibody (1:1,000) overnight at 4°C. After washing in PBS, slides were incubated with biotinylated goat anti-rabbit IgG and subsequently with VECTASTAIN ABC reagents (Vector Laboratories) according to the manufacturer’s instructions. Paip2, Pabp, and Tp2 were detected with DAB Peroxidase Substrate Kit (Vector Laboratories) according to the manufacturer’s instructions and then counterstained with hematoxylin. Pictures were taken using a CoolSnap camera attached to a Leica DM LB2 microscope.

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In situ hybridization. Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer’s protocol. RNA samples were separated on 4.5% acrylamide–50% (w/v) urea gel using TBE (89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA [pH 8.9]) as a running buffer. RNAs were electrophoresed onto an Amersham Hybnd-N (GE Healthcare, RPN303B). The probes were generated by RT-PCR using total testis RNA at 1:1,000.
RNA. The plots were incubated with the probes labeled with [32P]dCTP using Amersham Ready-To-Go DNA Labeling Beads (–dCTP) (GE Healthcare) according to manufacturer’s instructions. The signal was detected by autoradiography.

RNAse H treatment. Total testis RNA (5 µg) was incubated with RNase H (New England BioLabs Inc.) in the presence or absence of Oligo(dT)12-18 Primer (Invitrogen) according to the manufacturer’s instructions. After phenol-chloroform extraction, RNA samples were subjected to Northern blot analysis.

In vitro translation. Translation of capped Luc(Ava) mRNA in Krebs-2 extract or Pabp-depleted Krebs-2 extract was performed as previously described (39). Luc activity was measured by a Lumat LB 9507 bioluminometer (EG&G Bertold).

Chemical crosslinking assay. The 3’ poly(A) tail of uncapped Luc mRNA (Promega) was extended using a Poly(A) Tailing Kit (Ambion). Luc(A+) mRNA was radioactively labeled at the 5’ end using vaccinia virus guanylyltransferase (Ambion) with [α-32P]GTP (PerkinElmer) in the presence or absence of Oligo(dT)12-18 Primer (Invitrogen) according to the manufacturer’s instructions. After oxidation with NaIO4, the cap-labeled Luc(A+) mRNA was used for chemical crosslinking studies in RRL (Promega) as described previously (41, 63). Paip2a/Paip2b-depleted RRL was prepared as described (39).

Statistics. The statistical significance of the difference between the mean values for the different genotypes was evaluated using 2-tailed, unpaired Student’s t-test. The data were considered significant when the P-value was less than 0.05.

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35. Berlanga JJ, Baas A, Sonenberg N. Regulation of poly(A) binding protein function in translation.
characterization of the Paip2 homolog, Paip2B.
36. Gray NK, Wicken M. Control of translation ini-
14:399–458.
37. Luitjens C, Gallegos M, Kraemer B, Kimble J, 
Wicken M. CPEB proteins control two key steps 
38. Gorgoni B, Gray NK. The roles of cytoplasmic 
poly(A)-binding proteins in regulating gene 
expression: a developmental perspective. Brief Funct 
39. Svitkin YV, Sonenberg N. An efficient system for 
cap- and poly(A)-dependent translation in vitro. 
40. Kahvejian A, Svitkin YV, Sukaneh R, M’Boutchou 
MN, Sonenberg N. Mammalian poly(A)-binding 
protein is a eukaryotic translation initiation fac-
tor, which acts via multiple mechanisms. Genes Dev. 
41. Svitkin YV, et al. General RNA-binding proteins 
have a function in poly(A)-binding protein-depen-
42. Yanagiya A, Svitkin YV, Shibata S, Mikami S, 
Imataka H, Sonenberg N. Requirement of RNA 
binding of mammalian eukaryotic translation 
initiation factor 4G (eIF4G) for efficient inter-
action of eIF4E with the mRNA cap. Mol Cell Biol. 
43. Jacobson A. Poly(A) metabolism and translation: 
the closed-loop model. In: Hershey JW, Mathews 
MB, Sonenberg N, eds. Translational Control. Wood-
bury, New York, USA: Cold Spring Harbor Labora-
44. Burd CG, Matunis EL, Dreyfuss G. The multiple 
RNA-binding domains of the mRNA poly(A)-bind-
ing protein have different RNA-binding activities. 
45. Gorlach M, Burd CG, Dreyfuss G. The mRNA 
poly(A)-binding protein: localization, abundance, 
and RNA-binding specificity. Exp Cell Res. 1994; 
211(2):400–407.
46. Afonina E, Neumann M, Pavlakis GN. Preferential 
binding of poly(A)-binding protein 1 to an inhibi-
tory RNA element in the human immunodefi-
ciency virus type 1 gag mRNA. J Biol Chem. 1997; 
47. Kuhn U, Pieler T. Xenopus poly(A) binding protein: 
functional domains in RNA binding and protein-
48. Deo RC, Bonanno JB, Sonenberg N, Burley SK. 
Recognition of polyadenylate RNA by the poly(A)- 
49. Mruk DD, Cheng CY. Sertoli-Sertoli and Sertoli-
germ cell interactions and their significance in germ 
cell movement in the seminiferous epithelium during 
50. Mruk DD, Cheng CY. Cell-cell interactions at the 
eccoplasmic specialization in the testis. Trends 
51. Matzuk MM, Lamb DJ. The biology of infertility: 
research advances and clinical challenges. Nat Med. 
52. O’Bryan MK, et al. Sox8 is a critical regulator of 
adult Sertoli cell function and male fertility. Dev 
taxiCIF-64 causes spermatogenic defects and male 
54. Yoshida M, et al. Poly(A) binding protein (PABP) 
homeostasis is mediated by the stability of its 
inhibitor, Paip2. EMBO J. 2006;25(9):1934–1944.
55. Liu P, Jenkins NA, Copeland NG. A highly effi-
cient recombinant-based method for generat-
ing conditional knockout mutations. Genome Res. 
Hoxa-1.3 retinoic acid response element (3RARE) 
57. Stankar LH, et al. Immunological evidence for a 
P2 protamine precursor in mature rat sperm. Mol 
58. Heidarani MA, Kozak CA, Kistler WS. Nucleotide 
sequence of the Stp-1 gene coding for rat spermatid 
nuclear transition protein 1 (TP1): homology with 
protamine P1 and assignment of the mouse Stp-1 
59. Green GR, Balhorn R, Poccia DL, Hecht NB. Synthesis 
and processing of mammalian protamines and tran-
60. Belfve AR, Millette CF, Bhatnagar YM, O’Brien DA. 
Dissociation of the mouse testis and characteriza-
tion of isolated spermatogenic cells. J Histochem 
61. Platz RD, Mestrich ML, Grimes SR Jr. Low-molec-
ular-weight basic proteins in spermatids. Methods 
protein 2 gene affects sperm chromatin structure 
and reduces fertility in mice. Mol Cell Biol. 2001; 
21(22):7243–7255.
63. Sonenberg N. ATP/Mg++-dependent cross-linking 
of cap binding proteins to the 5′ end of eukaryotic 