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Epab Is Dispensable for Mouse Spermatogenesis and Male Fertility

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During spermatogenesis, gene expression is regulated post-transcriptionally by modification of the poly-adenine (poly(A)) tail length of repressed mRNAs. In a previous study, we demonstrated that the expression of two mRNA binding proteins, embryonic poly(A)-binding protein (EPAB), and somatic cytoplasmic poly(A)-binding protein (PABPC1), is spatially and temporally controlled during male germ-cell development, suggesting that they may modulate gene expression in different spermatogenic cell types (Ozturk et al., 2012).

We therefore evaluated the fertility of homozygous *Epab* knockout male mice (*Epab*^{-/-}) to further define the importance of EPAB during spermatogenesis. *Epab*^{-/-} mice were generated by targeted deletion of *Epab* exon 2, as described previously (Guzeloglu-Kayisli et al., 2012). The absence of exon 2 generates a stop codon in exon 3, and consequently encodes a truncated EPAB protein product that contains only part of the first RNA recognition motif (RRM) and lacks the remaining three RRM and the PAPC domain. Reverse-transcription PCR (Fig. 1A) and RNA in situ hybridization (not shown) were used to confirm the absence of full-length *Epab* transcripts in the testis of *Epab*^{-/-} males. *Epab*^{-/-} males are viable, phenotypically normal, and do not exhibit differences in weight as compared to their wild-type (*Epab*^{+/+}), and heterozygous knockout (*Epab*^{+/-}) littermates.

The fertility of *Epab*^{+/+}, *Epab*^{+/-}, and *Epab*^{-/-} male mice was tested by mating with wild-type females of proven fertility (male:female ratio of 1:2), for a total of 20 weeks (minimum of four oestrus cycles with each male). *Epab*^{-/-} male mice produced a similar number of pups compared to wild-type and heterozygous breeding males. Additional fertility parameters—including, testis weight, sperm count, and morphology, motility, and apoptosis index—were all normal in *Epab*^{-/-} males (data not shown).

Furthermore, *Epab*^{-/-} males exhibit normal seminiferous tubule structure and complete spermatogenesis, as determined by histological analysis (Fig. 1B–D). In contrast to its essential role in female fertility (Guzeloglu-Kayisli et al., 2012), these results demonstrate that EPAB is not required for spermatogenic functions in male mice.

It is possible that *Epab* is important for male fertility, but its loss is compensated for by the action of other RNA binding proteins, such as MSY2, DAZL, PUM2, or PAPBC1. We therefore used quantitative reverse-transcription PCR to measure the transcript levels of these RNA binding proteins, as well as the kinase *c-Mos*, in the testes of *Epab*^{+/+} and *Epab*^{-/-} males (Fig. 1E). We found that *Msy2* mRNA expression increased significantly by 50% as a result of *Epab* deletion ($P < 0.05$). *Msy2* is exclusively expressed in germ cells, is essential for male and female fertility (Yang et al., 2005), and could compensate for the loss of *Epab*. Yet MSY2 protein levels were similar in *Epab*^{+/+} and *Epab*^{-/-} male testes, as observed for the other proteins examined.

In summary, our findings demonstrate that EPAB is dispensable for male fertility in mice. Although the expression of *Epab* is spatially and temporally regulated during spermatogenesis, we did not detect any defects in male fertility parameters in *Epab*^{-/-} males as compared to wild-type males. We conclude that either EPAB is not important for male fertility, or that the phenotype associated with the loss of EPAB is masked by another protein. Since post-transcriptional gene expression is essential at several steps during male germ-cell development, it is likely that multiple, redundant mechanisms have evolved to control translational activation and repression.

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Figure 1.

EPAB is dispensable for spermatogenesis. **A:** PCR amplification of *Epab* from cDNA confirm that exon 2 is properly deleted in *Epab*^{-/-} testes. β -Actin was used as a loading control. **B:** The percentage of sperm cells with head, mid-piece, and tail defects. Results are presented as the mean $\pm$ standard deviation. **C:** Representative image of hematoxyline- and eosine-stained *Epab*^{-/-} testis section. Scale bar, 25 mm. **D:** The number of germinal epithelial cells in the seminiferous tubules of *Epab*^{+/+} and *Epab*^{-/-} male testes (spermatogonia (SG), spermatocytes (SC), round spermatid (RS), elongated spermatid (ES), Sertoli cell (ST)). Results are presented as the mean $\pm$ standard deviation. **E:** mRNA expression of *Msy2*, *Pum2*, *Dazl*, *Pabpc1*, *c-Mos*, and *Paip2* from *Epab*^{+/+} and *Epab*^{-/-} testes using quantitative reverse-transcriptase PCR, and normalized to β -actin. * Indicates statistical significance, as determined by Mann-Whitney U-test ($P < 0.05$). Results are presented as fold change standard deviation. **F:** *Msy* protein levels in testis lysate by Western blot analysis. Bands were quantified using densitometry, and normalized to GAPDH.