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RESEARCH HIGHLIGHT

Spermatids do it differently! Paip2a—the essential regulator of spermiogenesis?

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The mechanisms underpinning the latter stages of spermiogenesis are poorly understood and male germ cells have been presumed to extensively employ post-transcriptional regulatory machinery, in order to produce the highly differentiated spermatozoa, in the absence of newly synthesized gene transcripts. Excitingly, in a recently published paper in the *Journal of Clinical Investigation*,[1] two groups at McGill University, using null mouse models, have identified a crucial role of the poly(A)-binding protein-interacting protein 2 (Paip2a), in translational activation and protein homeostasis in the transcriptionally quiescent and terminally differentiating elongating spermatids.

Synchronized mRNA translation has emerged as a pivotal mechanism controlling temporal and spatial gene expression in cells and thus maintaining normal cellular and developmental processes.[2] This post-transcriptional regulatory machinery governs both mRNA stability and mRNA translation, with the target mRNAs subjected to enhancement, degradation or repressed from undergoing translation until specifically required.[3] In gametogenesis, premeiotic transcription and subsequent translational repression is a common mechanism throughout metazoans, with large numbers of genes identified that are translated post-meiotically in a variety of organisms from fly to man.[4,5] In the mammalian testis, several RNA-binding proteins including DAZL, RBYM, MSY2 and SAM68 have been linked to post-transcriptional control of gene expression in developing male germ cells.[6]

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The most well-understood system of translational delay occurs in the developing oocyte. Following export into the cytoplasm, specific 3′-untranslated region elements target critical maternal mRNAs for rapid poly(A) tail shortening, thus ensuring stabilization and transcriptional silencing (Figure 1a). When required, these transcripts undergo rapid repolyadenylation within the cytoplasm and a third of mouse and human oocyte mRNAs are regulated in this fashion (Figure 1b). Incorporated into these cytoplasmically polyadenylated mRNAs are *cis*-acting sequences that interact with crucial polyadenylation cofactors and collectively positively and negatively control polyadenylation and ultimately the translational activity of regulated mRNAs.

Cytoplasmic polyadenylation-binding protein (PABP) is a unique translation initiation factor in that it binds to the mRNA 3' poly(A) tail (Figure 1b) and stimulates recruitment of the ribosome to the 5' end of the mRNA (Figure 1c). PABP may also interact with the polyadenylation complex components, thereby stimulating translation and protect-

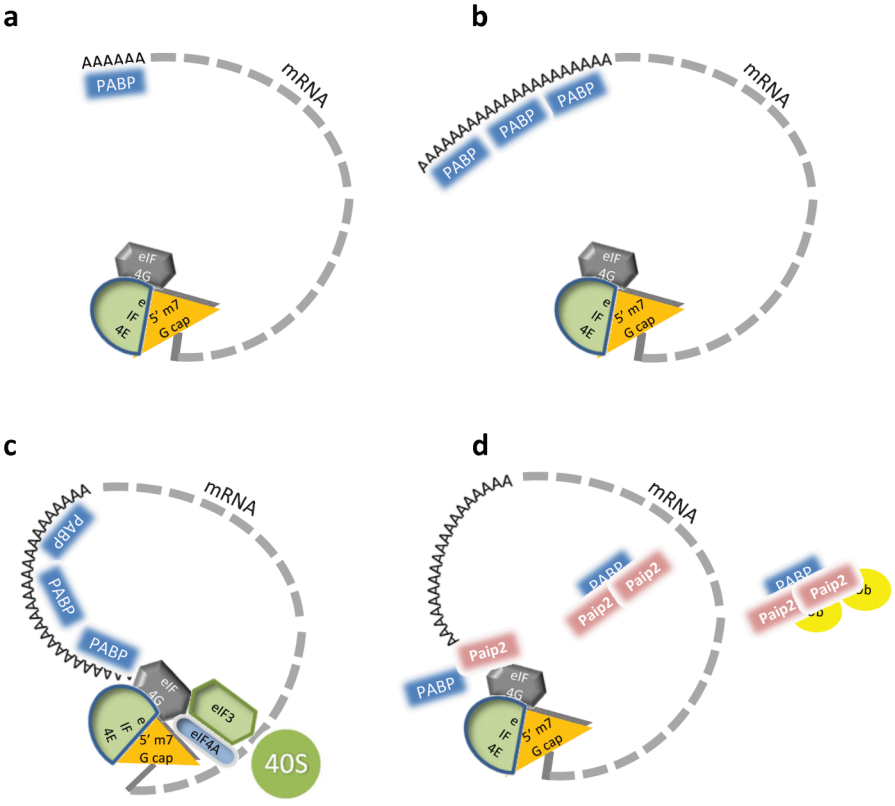

Figure 1 Control of translational activation in the developing oocyte. (**a**) Translation is repressed; (**b**) cytoplasmic poly(A) tail re-adenylation; (**c**) translation is correctly activated; (**d**) translation is correctly halted.

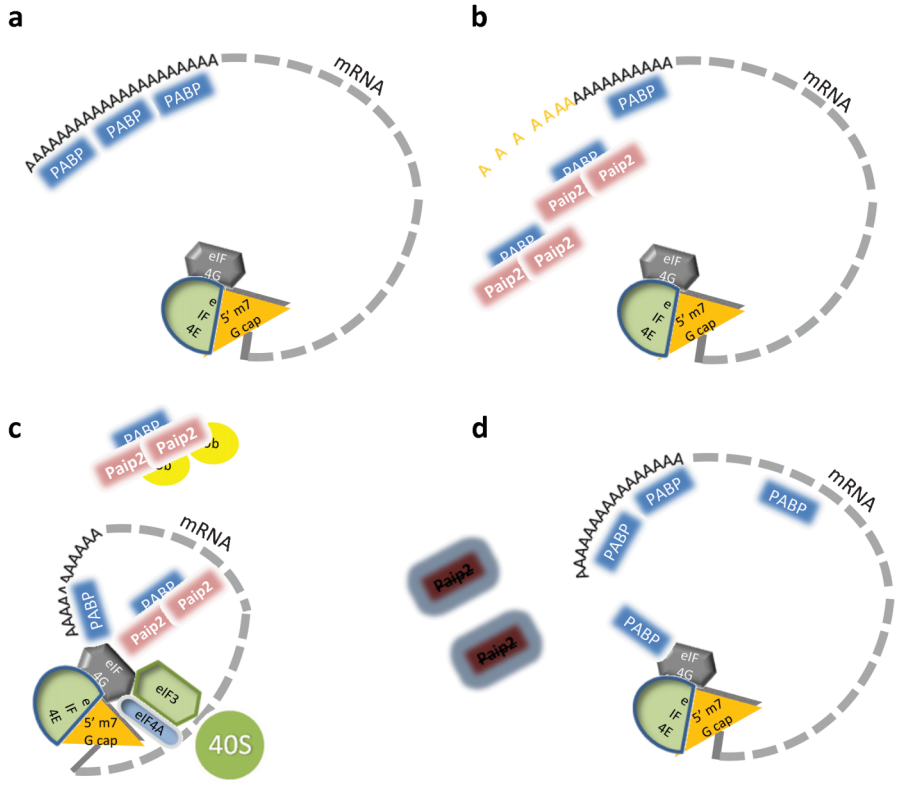

Figure 2 Control of translational activation in the differentiating spermatid. (**a**) translation is repressed; (**b**) cytoplasmic poly(A) tail de-adenylation; (**c**) translation is correctly activated; (**d**) translation is incorrectly repressed in the Paip2a null mouse. Paip2a, poly(A)-binding protein-interacting protein 2a.

ing against deadenylation (Figure 1c). Three PABP-interacting proteins (Paips) regulate PABP activity. Paip1 interacts with eukaryotic initiation factor 4A (eIF4A) and eIF3 to stimulate translation.[7] In contrast, Paip2a and Paip2b act as translational inhibitors.[7] Notably PABP activity is restricted by Paip2a, which inhibits translation by displacing PABP from the mRNA and competes with eIF4G for PABP binding (Figure 1d). This is a dynamic homeostasis, because, as PABP is reduced, Paip2a is ubiquitinated and degraded, allowing PABP to re-instigate translational activation.[8]

Less well characterized are the functions of PABP and its regulators in spermiogenesis. Two isoforms of cytoplasmic PABP exist in the mouse testis; PABPC1 and a testis-specific PABPC2, are present in pachytene spermatocytes and round spermatids, with PABPC1 also expressed in elongating spermatids. Both cytoplasmic isoforms can enhance translation *in vitro* but localisation studies have led to speculation that PABPC2 functions primarily as a translational repressor during late spermatogenesis.[9] The translational inhibitor, Paip2b, is minimally expressed in the testes. In contrast, Paip2a was known to be highly expressed in the testes and in this study was definitively localized to the cytoplasm of spermatids in the latter stages of spermiogenesis.[1]

The McGill groups then generated *Paip2a*-knockout (KO), *Paip2b*-KO, and *Paip2a/Paip2b*-double-KO mice and demonstrated that *Paip2a* null males and *Paip2a/Paip2b*-double-KO males are infertile with aberrant germ cell morphogenesis in late spermatogenesis and impaired spermiation. This is not unprecedented as multiple members of the PABP family and its interacting proteins exist in various organisms, with some having essential testis specific roles, e.g., *Drosophila* eIF4G2 mutants are male sterile and the testes contain spermatocytes but lack elongated spermatids or mature sperm.[10] In these studies, neither Paip2 KO affects female fertility, suggesting that the removal of PABP in the oocyte may also be attributable to other factors.

Strikingly, however, the levels of three key spermatid chromatin remodelling proteins (protamine 1, transition proteins 1 and 2) were markedly reduced in the *Paip2a/Paip2b* double KO. This is at odds with the proposed canonical role of Paip2a as a translational inhibitor (Figure 1d), as logically in the absence of Paip2a, translation of PABP-targeted mRNA should be enhanced! In recent work, Kimura and colleagues[9] suggest that PABPC2 may be required for protection of haploid-specific mRNAs against precocious translation in round spermatids. This led the McGill group to further investigate the alternative strategy adopted in spermiogenesis, where extended poly(A) tails on stored mRNAs contribute to transcript stability and translational inhibition (Figure 2a) *via* binding of PABPC2. Poly(A) tail shortening and the subsequent sequestering and ubiquitination of displaced PABP by Paip2a (Figure 2b), results in the formation of a translational initiation complex (Figure 2c), and the correct production of key proteins. In the absence of Paip2a, the resultant excessive levels of PABP production and degradation prevent accurate morphogenic protein translation (Figure 2d) resulting in abnormal spermiogenesis and defective spermiation in the null testis.

This study highlights that, in addition to controlling spatial and temporal production of target proteins, RNA-binding proteins are crucial to the maintenance of correct stoichiometric ratios of proteins, and that excessive levels in cells can result in loss of specificity and off-target promiscuity. Corroboration of these findings can be found in earlier studies of overexpression of *Drosophila* Paip2 which results in the inhibition of cell growth. This can be counteracted by co-overexpressing *Drosophila* PABP and restoring the balance of activator and inhibitor.[11] In supporting observations, complete loss of *Drosophila* PABP in flies causes cell lethality due to G_2 arrest but mutant flies with hypomorphic alleles are viable and the males sterile due to defects in meiotic spindle formation, chromosome segregation and cytokinesis.[12]

In summary, these observations reveal that strict control over levels of PABP in germ cells is crucial to normal sperm production and release—and optimal PABP levels exist within a tightly regulated concentration range. Interactions between transcription and translation demonstrate that PABP and Paip2a do in fact operate to regulate each other's cellular abundance—resulting in a crucial molecular switch that turns on among others, vital chromatin packaging proteins at the correct point in sperm cell differentiation.

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