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Prospects & Overviews

Revisiting poly(A)-binding proteins: Multifaceted regulators during gametogenesis and early embryogenesis

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Abstract

Post-transcriptional regulation faces a distinctive challenge in gametes. Transcription is limited when the germ cells enter the division phase due to condensed chromatin, while gene expression during gamete maturation, fertilization, and early cleavage depends on existing mRNA post-transcriptional coordination. The dynamics of the 3′-poly(A) tail play crucial roles in defining mRNA fate. The 3′-poly(A) tail is covered with poly(A)-binding proteins (PABPs) that help to mediate mRNA metabolism and recent work has shed light on the number and function of germ cell-specific expressed PABPs. There are two structurally different PABP groups distinguished by their cytoplasmic and nuclear localization. Both lack catalytic activity but are coupled with various roles through their interaction with multifunctional partners during mRNA metabolism. Here, we present a synopsis of PABP function during gametogenesis and early embryogenesis and describe both conventional and current models of the functions and regulation of PABPs, with an emphasis on the physiological significance of how germ cell-specific PABPs potentially affect human fertility.

KEYWORDS
early embryogenesis, fertility, gametogenesis, poly(A)-binding proteins, post-transcriptional regulation

INTRODUCTION

The fertilization of an egg by a sperm marks the potential of a new life. This is a long, well-planned encounter prepared, even before birth. After fertilization, the totipotent zygotes undergo preimplantation development and implant into the uterine wall to create a unique individual. In mice, primordial germ cells (PGCs) form around embryonic day 7.25 (E7.25).[1] The PGCs migrate to and colonize the genital ridge, and then proliferate during their migration to the gonads (E7.75–E10.5).[2] Following the sex determination of the embryo (~ E13.5), the PGCs start to accept differentiated fates and form spermatogonia and oogonia.[3] The male germ cells enter a quiescent state after mitotic arrest in the G0/G1 phase, whereas female germ cells start the meiotic process and halt at the diplotene stage of prophase I, forming primordial follicles together with surrounding squamous pre-granulosa cells.[4]

Spermatogenesis consists of three parts: spermatocytogenesis, meiosis, and spermiogenesis.[5] During spermatocytogenesis, a portion of spermatogonial stem cells (SSCs) restore the spermatogonial pool through a succession of mitotic divisions, while the rest differentiate and enter the meiotic stage to form primary spermatocytes. After two meiotic divisions, haploid spermatids are produced. Following morphological and physiological transformation, the cells enter the spermiogenesis stage, which includes nuclear condensation, flagella, and acrosome formation, and mitochondrial restructuration to form spermatozoa.[6,7]

In contrast to the unlimited generation of sperm,[8] there is a numerically defined oocyte pool in mammalian females. A subgroup of primordial follicles is activated in a gonadotropin-independent manner,

Abbreviations: APA, alternative cleavage and polyadenylation; COC, cumulus oocyte complex; DSB, double-strand break; GV, germinal vesicle; KO, knockout; MZT, Maternal-to-zygotic transition; NMD, nonsense-mediated decay; PABP, poly(A)-binding protein; PAIP, PABP-interacting protein; PGC, primordial germ cell; RRM, RNA recognition motif; SSC, spermatogonial stem cell; ZGA, zygotic genome activation

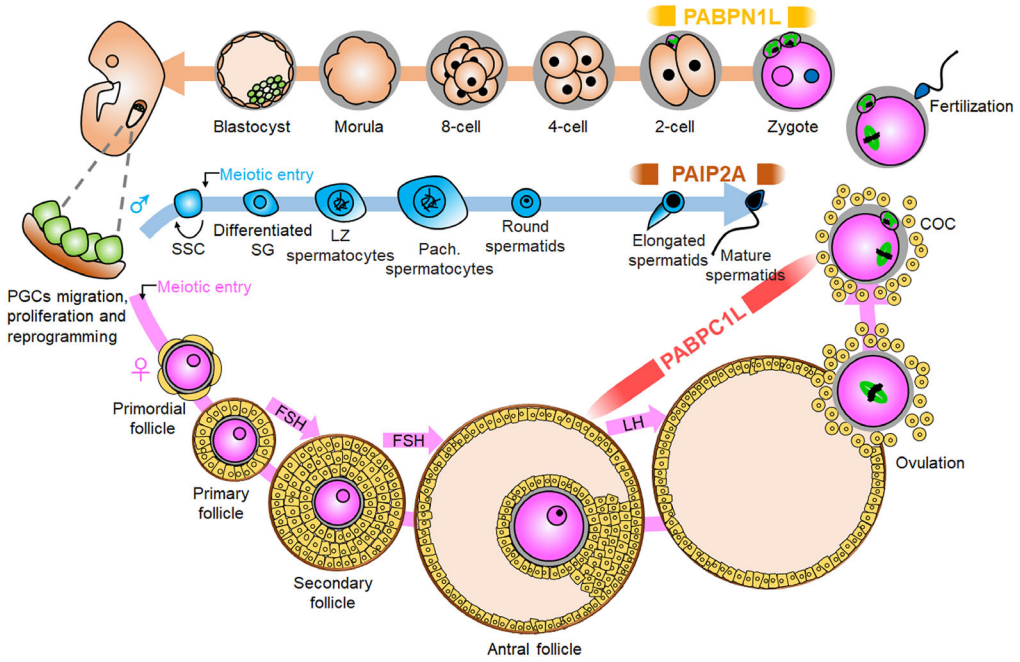

FIGURE 1 Gametogenesis and early embryogenesis in mice and the effects of *Pabpc1l*, *Pabpn1l*, and *Paip2a* knockout. Primordial germ cells (PGCs) emerge in post-implantation embryos, proliferate, and migrate towards the genital ridge. In males, PGCs develop into prospermatogonia and enter mitotic arrest. After birth, prospermatogonia resume mitotic proliferation and form spermatogonia. A subset of spermatogonia enters meiosis and further differentiates into spermatocytes, whereas the others serve as spermatogonial stem cells (SSCs) that replenish the spermatogonial pool. After meiosis completion, spermatocytes form round spermatids. Following morphological changes, including chromatin condensation and cellular elongation, the mature spermatids are produced. In females, PGCs directly enter and arrest at prophase in the first meiosis, associating with the surrounding pre-granulosa cells to form primordial follicles. Upon puberty, the primordial follicles begin oogenesis. The squamous pre-granulosa cells surrounding the oocyte in the primordial follicles transform into cuboidal granulosa cells, to form primary follicles. Theca cells aggregate to form an outer layer of the oocyte. Granulosa cells proliferate, increase in layers surrounding the oocyte, which enables the follicle to reach the secondary follicle stage. The antral follicles appear, distinguished by a fluid-filled antral cavity. The oocyte grows into the fully grown stage, increasing in size and acquiring meiosis competency. Thereafter, luteinizing hormone (LH) stimulates ovulation: cumulus granulosa cells surrounding the oocyte expand, the oocyte resumes meiosis, undergoes nuclear envelope breakdown, spindle assembly, and the first polar body extrusion. The oocyte finally arrests in metaphase II until fertilization. Upon sperm binding, the egg completes meiosis and expels the second polar body, forming the zygote. After the first cleavage division, the embryo finishes its maternal-to-zygotic transition (MZT). Following continued mitotic divisions, the embryo undergoes compaction to form the morula and develops a fluid-filled cavity of the blastocyst. The *Pabpc1l*, *Pabpn1l*, and *Paip2a* knockout mice models revealed their indispensable roles during gametogenesis and MZT. The time frame corresponding to the observed phenotypes of the indicated genes are shown in the schematic. LZ, leptotene/zygotene; Pach., pachytene.

while pre-granulosa cells are converted into cuboidal cells and develop into primary follicles. After successive mitotic divisions, granulosa cells accumulate into multiple layers to form secondary follicles. Follicle-stimulating hormone (FSH) receptors are expressed in the granulosa and theca cells in the secondary follicle stage, which promote subsequent gonadotropin-dependent follicular development.[9] Accompanied by the appearance of a big antrum in the follicles, the oocytes surrounded by cumulus cells are pushed to the other side of the follicles to form antral follicles. During these stages, the oocytes increase in size and synthesize many dormant mRNAs used in meiotic maturation and early embryo development. However, transcription is silenced as the oocytes arrive at the fully grown stage in antral follicles. Upon luteinizing hormone (LH) stimulation, oocyte meiotic activity resumes as the nucleus breaks down and the first polar body extrudes. The oocytes are arrested again and ovulated combined with surrounding cumulus cells, now called cumulus oocyte complexes (COCs), pending fertilization.[10]

After germ cell specification, the male and female gametes fuse to form the zygote, during which the oocytes complete meiosis II and release a second polar body. Maternal-to-zygotic transition (MZT) also occurs, during which developmental control is passed from maternal materials to the products of the zygotic genome. MZT has two key features: (1) the maternal mRNAs are degraded and (2) the zygotic genome is transcriptionally activated (ZGA).[11] Undergoing several mitotic cell divisions, the blastomeres establish adhesion between each other, hatch out of the zona pellucida, and implant into the uterine wall.[12] Gametogenesis and early embryogenesis are graphically summarized in Figure 1.

Development through gametogenesis, MZT, and early embryogenesis is a unique and significant cellular transition. During the transition, transcriptional activity is highly dynamic and coupled with the developmental stage. In males, chromatin remodeling is an essential process during spermiogenesis that drives the nucleosome from a

histone-surrounded structure to a largely protamine-based assembly. The tightly packed DNA-protamine complexes halt transcription during spermiogenesis.[13,14] In the oocyte, the transcriptional activity stops once oocytes arrive at the fully grown stage. Succeeded by meiotic resumption, fertilization, and the first embryonic division, the 2-cell embryos initiate ZGA. Multiple biological processes substantially take place in the absence of new transcripts. Therefore, the timely and selective translation and degradation of the preserved transcripts are necessary for these well-organized activities.

Post-transcriptional regulation opens up possibilities to meet these requirements. RNA tailing is the addition of non-template nucleotides to the 3′-end of RNA. This is the most frequent post-transcriptional modification and poly adenosine (poly(A)) tails are key players in mRNA stability control. The deadenylation of mRNA leads to RNA degradation, while polyadenylation facilitates mRNA stability and translatability.[15] Additionally, a number of specialized poly(A)-binding proteins (PABPs) make up an orchestrated mRNA regulatory system. They adorn mRNA poly(A) tails in the nucleus and remain during translation of the mRNA into the cytoplasm. PABPs do not have any catalytic domains but function as scaffolds for proteins and RNAs that mediate poly(A) elongation and shortening. There are recent reports of germ cell-specific PABP members that broaden the roles of PABPs in multiple cellular processes.[16–19] In this review, we propose novel hypotheses of the molecular mechanism and physiological significance of PABPs during germ cell development and MZT, particularly in mouse models.

CATEGORY AND STRUCTURE OF PABPs

Poly(A)-binding proteins (PABPs) have been identified in eukaryotes but not in prokaryotes. The category of PABPs varies with the organism but can be organized into two structurally distinct groups based on their subcellular localization and phylogeny: (1) cytoplasmic PABPs (PABPCs), including PABPC1, PABPC3, PABPC4, PABPC4L, the X chromosome encoded protein PABPC5, and PABPC1-like (PABPC1L, also known as embryonic PABP [ePAB]); and (2) nuclear PABPs (PABPNs), including PABPN1, and PABPN1-like (PABPN1L, also known as embryonic PABP2 [ePABP2]).[20]

Canonical PABPCs possess four highly conserved RNA recognition motifs (RRMs) and a C-terminal PABP domain[20,21] (Figure 2A). It was shown that PABPC requires a minimum binding site of 12 adenosines and is saturated by about 25 adenosines in vitro.[22,23] In contrast, the PABP domain mediates self-interaction and association with other proteins, such as PABP-interacting proteins (PAIPs), which are critical for regulating PABP activity[24] eukaryotic translation termination factor 3 (eRF3), and deadenylases.[25]

PABPNs have a single RRM, a long acidic N-terminus, and a short arginine-rich C-terminus[26] (Figure 2A). The N-terminal domain facilitates poly(A) polymerase-mediated polyadenylation by direct interaction with poly(A) polymerase.[27] The RRM allows PABPN1 to occupy approximately 11–14 adenosines along the poly(A) tail.[28] The C-terminal domain contains asymmetrically dimethylated arginine residues and a nuclear localization signal (NLS), both of which are responsible for the nuclear accumulation of PABPN1.[29,30]

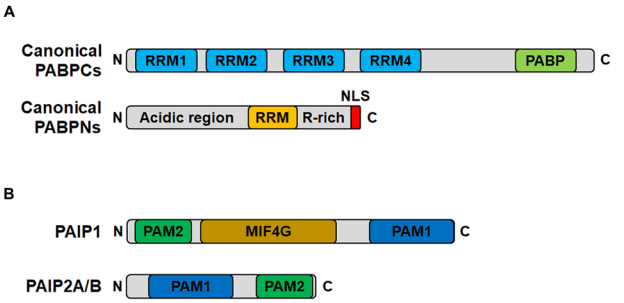

FIGURE 2 Structural features of the cytoplasmic and nuclear PABPs and PABP-interacting partners (PAIPs). **(A)** Cytoplasmic PABPs contain four different RNA recognition motifs (RRMs), a C-terminal PABP domain; while nuclear PABPs contain only one RRM domain, an acidic region at the N-terminal, an arginine (R)-rich domain, and a nuclear localization signal (NLS) at the C-terminus. **(B)** Two PAIPs have been identified: PAIP1 and PAIP2. PAIP1 has a unique MIF4G domain in its middle region and PABP-binding motifs (PAMs). PAIP2 contains two isoforms: PAIP2A and PAIP2B both have PAM1 and PAM2 domains, but with the inverse distribution. The illustration is based on mouse proteins.

CYTOPLASMIC POLY(A) BINDING PROTEINS

General function of PABPCs

A poly(A) tail is required to interact with the m^7GpppN cap at the 5′-end of the mRNA to form a "closed-loop" that facilitates translation.[31] Two subunits of the cap-binding complex, eIF4E and eIF4G, cover the cap and recruit the 40S ribosomal subunit, respectively. Indeed, eIF4G acts as the scaffold in this "closed-loop" model by interacting with both eIF4E and PABPC1 and this complex is required for facilitating the initial recruitment of the 40S subunit during mRNA translational initiation.[32,33]

The PABPC1-poly(A) band is not only restricted to mRNA stabilization but also characterized by facilitating deadenylation for mRNA rapid turnover. Early studies in yeast reported that poly(A) tail shortening was inhibited in *Pab1* (ortholog of mammalian *Pabpc1*)-*null* mutants, suggesting that deadenylation is PAB1-dependent, although the mechanism was not further characterized.[34] Recently, Webster et al. (2018) and Yi et al. (2018) dissected the different roles of PAB1/PABPC1 in the two catalytic subunits of the CCR4-NOT (CNOT) complex to show CNOT7- or CNOT6-dependent mRNA deadenylation. CNOT6 is activated by its interaction with PABPC1 and removes PABPC1-bound poly(A) tails. The free poly(A) tail was also removed by CNOT7 and inhibited by PABPC1. Biochemical results from pull-down assays showed that PABPC1 interacts with CNOT6 through its C-terminal portion but not CNOT7, which accounts for the specific stimulation of PABPC1 in CCR4-mediated mRNA deadenylation.[35,36]

The nonsense-mediated decay (NMD) pathway also includes PABPC1 function. The NMD is a conserved mRNA surveillance

mechanism that detects and degrades mRNAs that contain premature translation termination codons (PTCs).[37] This process is activated by premature translation termination, which results in the assembly of the surveillance complex composed of the central NMD protein UPF1, an ATP-dependent RNA helicase, eukaryotic translation termination factors eRF1 and eRF3, and mRNA degradation enzymes.[37] PABPC1 aids in recognizing PTC and suppressing NMD.[38] NMD evasion depends on the C-terminus of PABPC1, which interacts with the N-terminal part of eRF3.[39] This PABPC1-eRF3 interaction competitively suppresses UPF1 binding with eRF3 to inhibit NMD.[40] Furthermore, the PABPC1-eIF4G interaction circularizes the mRNA to further prevent NMD through the juxtaposition of the stop and start codons to facilitate translation re-initiation after translation termination.[41]

These findings demonstrated that PABPC1 participates in most of the cytoplasmic post-transcriptional regulatory processes, including mRNA stabilization, translation promotion, and mRNA degradation regulation, by directly or indirectly interacting with their related factors. However, coordinating these seemingly contradictory functions of PABPC1 during gene expression regulation requires further investigation.

The regulation of PABPCs

Because PABPC plays multiple roles in mRNA metabolism, it is important to control PABPC expression and activity. PABPC1 controls its own mRNA translation through a feedback mechanism in which PABPC1 binds to an adenosine-rich cis-element of its own mRNA 5′-untranslated region and inhibits its own translation.[42] The extracellular signal-regulated kinase (ERK) signaling pathway mediates the phosphorylation of translation initiation factors, such as eIF4E, which is important in regulating global mRNA translation. PABPC1 is also phosphorylated via the ERK signaling pathway, and phosphorylated PABPC1 has a high affinity for eIF4G, which enhances cap-dependent translation.[43]

In addition to signaling cascade-mediated modification, PABPC activity is also closely related to PABP-interacting proteins (PAIPs). Two PAIPs have been identified: PAIP1[44] and PAIP2[45] Homologs of *Paip2* were recently cloned and named *Paip2a*, and *Paip2b*.[46] PAIPs contain two distinct PABP-binding motifs (PAMs): PAM1 and PAM2. PAM1 is approximately 25 amino acids and binds to the RRM2 of PABPC1[47]; PAM2 has a conserved peptide sequence (L/P/F)X(P/V)XAXX(F/W)XP that recognizes the PABP domain of PABPC1.[25] Additionally, PAIP1 has a unique MIF4G domain that is similar to eIF4G[44] (Figure 2B). PAIP1 acts as a translational promoter that associates with the RNA helicase eIF4A and the 40S ribosome-binding factor eIF3 to assure the interaction of PABPC1 with eIF4G and stimulate translation.[44,48] In contrast, PAIP2A and PAIP2B function as translational repressors that reduce PABPC1 affinity for the poly(A) tail and interrupts the interaction between PABPC1 and eIF4G.[21,46] PABPC1 can stimulate translation termination through its interaction with eRF3 via its PABP domain.[49] PAIP1 and PAIP2 bind the same domain of PABPC1 and prevent translation termination at the premature termination codon. However, these regulations become insensitive after PABPC1 binds the poly(A) tail.[50]

The functions of PAIPs have also been evaluated in germ cells and their protein levels were analyzed during mouse spermatogenesis. PAIP1 is highly expressed in pachytene spermatocytes to elongate spermatids during mouse spermatogenesis,[51] while both PAIP2 proteins are expressed at low levels in pachytene spermatocytes and round spermatids. Although PAIP2A is abundantly expressed in elongated spermatids, PAIP2B is not[52] (Figure 3A). *Paip2a* knockout (KO), *Paip2b* KO, and *Paip2a*/*Paip2b* double KO mice were generated to study the roles played by PAIP2s in spermatogenesis. Corresponding with the presence of PAIP2A in the latter stages of spermiogenesis, *Paip2a*-deficient and *Paip2a*/*Paip2b* double KO males were infertile due to abnormal germ cell morphogenesis in late spermatogenesis and impaired spermiation, but the loss of both *Paip2* homologs showed no effect on female fertility[52] (Table 1 and Figure 1). PABPC1 levels decreased during normal spermiogenesis, but the loss of PAIP2A led to a reciprocal increase in PABPC1 in elongating spermatids.[52] Aberrant accumulation of PABPC1 is thought to interfere with the translational activation pathway, which subsequently halts sperm development.[52] Although the mechanism by which PAIP2 regulates PABPC1 levels remains to be determined, regulation of PABPC1 levels in germ cells is needed for normal sperm production and release.

PABPCs in gametogenesis and early embryogenesis

PABPCs in spermatogenesis

In contrast to somatic cells, transcriptional activity does not always occur during gametogenesis. In male mice, transcriptional silencing occurs during spermatocyte meiotic entry and again during spermatid elongation and maturation, which involves histone exchange and altered chromatin compaction[53,54] (Figure 3A). The mRNAs are transcribed during male germ cell differentiation and deposited with long poly(A) tails, keeping them in a repressed state (Figure 3A).

A testis-specific PABP (tPABP; also known as PABPC2 in mice and PABPC3 in humans) was first detected in mice[55] and subsequently described in humans.[56] In mice, the *Pabpc2* gene is expressed in pachytene spermatocytes and round spermatids[17] (Figure 3A), while human *Pabpc3* mRNA is only present in round spermatids.[56] However, *Pabpc1* is also required in spermatogenesis and exhibits dynamic expression during mouse spermatogenesis. *Pabpc1* mRNA increases during the meiotic stage, peaking at the early post-meiotic stages, and is then undetectable until the completion of spermatogenesis.[57] Furthermore, PABPC1 associates with both translationally inactive monosomes and actively translating polyribosomes, whereas PABPC2 is only distributed in monosomes, suggesting distinct functions between PABPC1 and PABPC2.[17]

Indeed, both PABPC isoforms exist in pachytene spermatocytes and round spermatids, whereas only PABPC1 is present in elongated spermatids, which corresponds to the translational activation process[17] (Figure 3A). The mechanisms by which PABPC1 mediates mRNA

TABLE 1 Summary of PABPs and PAIPs involved in sperms, oocytes, and early embryos in mouse models

PABPs	Tissues	Subcellular localization	Knockout mouse		
			Phenotype	Targeted process	Developmental function
PABPC1	All[114]	Cytoplasm Nucleus[114,117]	Not determined	-	-
PABPC1L	Testis Ovary Early embryos[119]	Cytoplasm[119] Nucleus[75]	Male: fertile Female: infertile[119]	Failure to produce mature eggs[119]	Maternal mRNA translational activation[119]
				Smaller in size, containing peripheral germinal vesicles, and loosely associated with cumulus cells[76]	Chromatin reorganization and transcriptional silencing[76]
				Defective cumulus expansion[77]	Regulating cumulus and granulosa cell functions[77]
				Disrupted transzonal processes (TZPs) and gap junctions at the preantral stage of folliculogenesis[74]	Maintaining oocyte-somatic communication[74]
PABPC2	Testis[17]	Cytoplasm[17,75]	Male: fertile[58]	No effect[58]	No effect[58]
PABPN1	All[114]	Cytoplasm Nucleus[114]	Not determined	-	-
PABPN1L	Ovary Early embryos[19]	Cytoplasm Nucleus[19]	Male: fertile Female: fertile[19]	Maternal-to-zygotic transition (MZT)[19]	Maternal mRNA decay and stabilizing BTG4 protein[19]
PAIP1	All[24]	Cytoplasm[24]	Not determined	-	-
PAIP2A	All[24,52]	Cytoplasm[24,52] Nucleus[75]	Male: infertile Female: fertile[52]	Aberrant germ cell morphogenesis in late spermatogenesis and impaired spermiation[52]	Promoting efficient translation by inhibiting PABPC1 accumulation in late spermiogenesis[52]

Factors that are expressed in sperms, oocytes, or early embryos. The emphasized functions of the indicated factor are based on studies of the corresponding gene knockout mice.

translation during this process are largely explained by its binding partner PAIP2A in a KO mouse model. PAIP2A and PAIP2B are characterized as PABP modulators, with *Paip2a* single and *Paip2a* and *Paip2b* double KOs inhibiting the translation of a few mRNAs that encode proteins required for germ cell development. A moderate increase in PABPC1 protein levels in elongated spermatids was observed in *Paip2a* KO mice[52] (Table 1). Given that PABPC1 is upregulated in the *Paip2a* KO, the authors suggested that these abnormal high levels of PABPC1 inhibited translation. This hypothesis was supported by an in vitro assay that showed translation inhibited by high levels of recombinant PABPC1 could be restored with PAIP2A supplementation.[52] These findings proposed that a surplus of free PABPC1 could interact with eIF4G that competed with poly(A)-bound PABPC1 to disrupt mRNA circularization-mediated translation. The male germ cell-specific mechanisms of translational activation and temporal linkage to the function of PABPC1 remain to be thoroughly explored.

To investigate the function of the testis-specific PABP, *Pabpc2* mutant mice were generated. Surprisingly, these mutant mice exhibited normal fertility with normal spermatogenesis and sperm mobility[58] (Table 1). PABPC1 and PABPC2 could both interact with mRNA poly(A) tails to promote reporter mRNA translation and combine with diverse translation-related factors, such as eIF4G1, PAIP1, PAIP2, and Piwi-like protein 1.[17] Therefore, it is possible that PABPC2 is functionally redundant with PABPC1.

PABPCs in oogenesis and early embryogenesis

Similar to males, oocytes completely halt their transcription as they arrive at the fully grown germinal vesicle (GV) stage and remain silent until the final stages of maturation[59,60] (Figure 3B). During oocyte growth, transcribed maternal mRNAs are stored in the cytoplasm with a short poly(A) tail, allowing them to be in a translationally-repressed state.[61] During oocyte maturation and fertilization, the mRNAs are translationally activated through poly(A) tail elongation[62,63] (Figure 3B). Poly(A) tail-modulated translational activation of these pre-existing mRNAs is vital for oocyte maturation and early embryo development. A specialized PABP called poly(A)-binding protein cytoplasmic

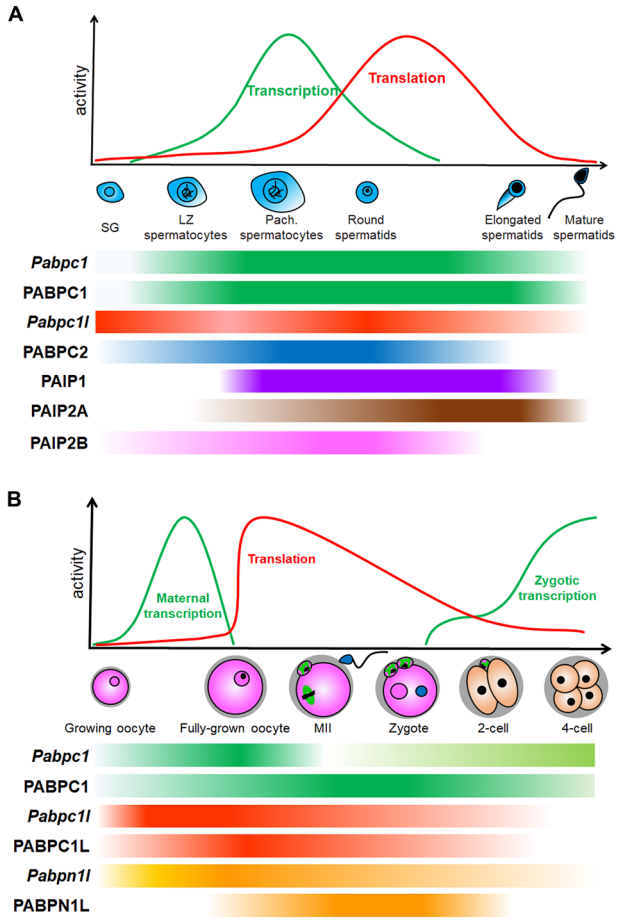

FIGURE 3 The expression levels of PABPs and PAIPs during gametogenesis and MZT in mice. (A) During spermatogenesis, transcription silencing occurs at meiotic entry and again during elongation of spermatids at mid-spermiogenesis and onward spermatogenesis. However, translation is persistent, regulating the timely expression of stored mRNAs during developmental stages. *Pabpc1*, *Pabpc1l*, and *Pabpc2* and their regulatory factors *Paip1*, *Paip2a*, *Paip2b* exhibit dynamic expression during spermatogenesis. **(B)** Transcription is turned off when the oocytes arrive at the fully grown stage and remains silent until zygotic genome activation (ZGA) at the later zygotic stage. Translational activation occurs following resumption oocyte meiosis and controls the expression of maternally-derived mRNAs during MZT. Developmental stage-specific expressions of *Pabpc1*, *Pabpc1l*, and *Pabpn1l* during oogenesis and MZT are shown. In both (**A**) and (**B**), the color gradient ribbons represent the expression levels of the indicated gene or protein, which are distinguished by italic and capital letters, respectively. Green and red curves represent transcriptional and translational activities, respectively. SSC, spermatogonial stem cell; LZ, leptotene/zygotene; Pach., pachytene; MII, metaphase II.

1 like (PABPC1L) plays a key role in these processes. PABPC1L was first characterized in *Xenopus*.[64] *Xenopus* oocyte maturation requires timely and strict protein expression, mainly through translational regulation of the pre-stored mRNA. Therefore, newly transcribed pre-mRNAs undergo a series of modifications including capping, splicing, and polyadenylation in the nucleus as observed in somatic cells. Along with transcription, pre-mRNAs are capped at the 5′-end[65] and then the cleavage and polyadenylation specificity factor (CPSF) complex binds the AAUAAA sequence (or the cleavage/polyadenylation site [PAS]) in the 3′-untranslated region (3′-UTR) and cleaves the transcript ~ 15–30 nucleotides downstream this signal. After that short poly(A) tails are attached at the 3′-end, and the molecule is translocated into the cytoplasm. In *Xenopus* oocytes, the activity of the deadenylase PARN was higher than that of cytoplasmic poly(A) polymerase GLD2[66]; therefore, a balance between PARN and GLD2 is needed to protect the stored mRNAs from PARN-mediated deadenylation and decay. The formation of the "closed-loop" (5′-caps-eukaryotic translation initiation factors-PABPC1-3′-tails present in somatic cells) is essential for protecting them from deadenylase attack and facilitating translation.[67] The remaining translation-related factors regulate translation through the "closed-loop" directly or indirectly in *Xenopus* oocytes.

The functions of PABPC1L during *Xenopus* oocyte maturation are characterized based on both polyadenylation-dependent and -independent pathways. For polyadenylation-related translation activation, PABPC1L covers the elongated poly(A) tails of mRNAs to stabilize them for further polyadenylation[66]; moreover, elongated poly(A) tail-associated PABPC1L promotes the dissociation of the translational suppressor Maskin from the cap-binding complex and promotes eIF4G-eIF4E interactions for the initiation of translation[68] (Figure 4A). In the polyadenylation-independent pathway, PABPC1L interacts with the 3′-UTR binding proteins PUM2 and DAZL, repressing premature translation in immature *Xenopus* oocytes.[69] This complex plays a critical role in stabilizing the stored mRNAs. In vitro progesterone-induced oocyte maturation led to the reversible removal of PUM2 from the complex, and the DAZL-PABPC1L complex activated mRNA translation[69] (Figure 4B). Moreover, PABPC1L was dynamically phosphorylated at four residues (Ser460, Ser461, Ser464, and Thr465) during *Xenopus* oocyte maturation; this hyperphosphorylated PABPC1L was able to bind to both translating mRNAs and the complex that is required for mRNA cytoplasmic polyadenylation and translation.[70] PABPC1L works with RNA-binding proteins associated with the 3-UTR cis-element and other translation-related factors during translation in *Xenopus* oocytes. Consistent with these roles of PABPC1L, the morpholino (the chemically synthesized oligomers that bind complementary target mRNAs and prevent their translation)-knockdown of PABPC1L causes *Xenopus* embryonic lethality, accompanied by anterior-posterior axis formation and movement defects.[71]

Pabpc1l, which was identified in mice, is transcribed in the ovary and testis (Figure 3A-B), but not in somatic tissues.[18,72] There are significant differences in the mRNA regulation mechanisms in *Xenopus* and mammals; for example, the role of GLD2 in regulating cytoplasmic polyadenylation has been well established in *Xenopus* oocytes, but *Gld2*-deficient mice show normal fertility with equal polyadenylation activity in oocyte extracts compared with the wild-type mice.[73] Moreover, no mammalian ortholog of Maskin has been reported. *Pabpc1l* mRNA levels are higher in oocytes from preantral follicles than in primary follicles and is expressed similarly in oocytes derived from preantral, early antral, and antral follicles, although it is not detected in the granulosa cells.[74] *Pabpc1l* mRNA is also

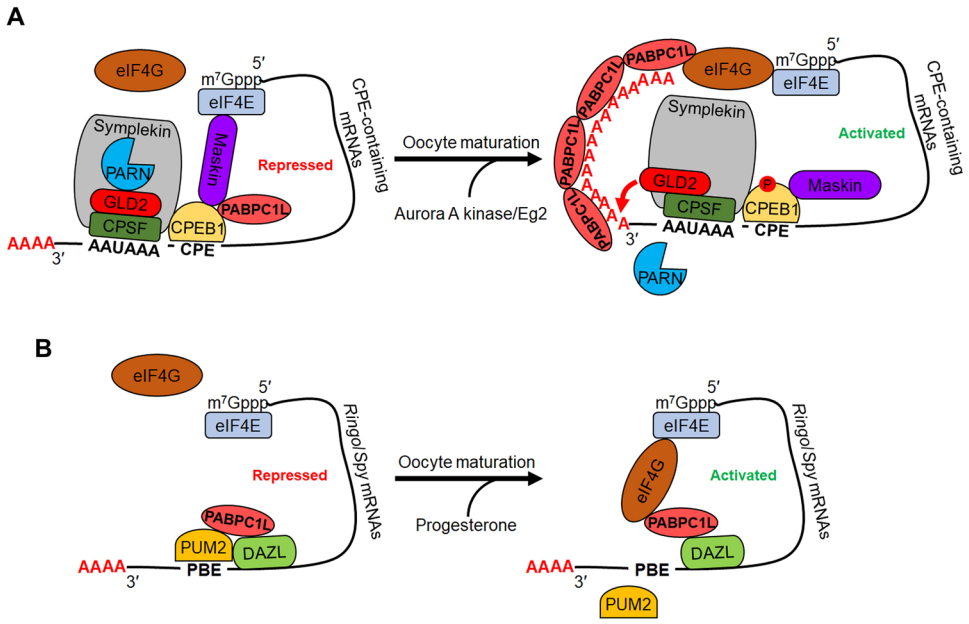

FIGURE 4 Translational activation and the role of PABPC1L in *Xenopus* oocytes. **(A)** Translation activation of the repressed cytoplasmic polyadenylation element (CPE)-containing mRNAs is triggered by Aurora A/Eg2-mediated CPE-binding protein 1 (CPEB1) phosphorylation. CPE is present on the 3′-UTR of CPE-containing mRNAs, acting as the binding platform for CPEB1. CPEB1 binds to the CPE on dormant mRNAs, forming a complex with CPSF, Symplekin, GLD2, and PARN. Maskin suppresses the translation of CPE-containing mRNAs by interacting with eIF4E to competitively inhibit eIF4G-eIF4E interactions, thereby blocking the assembly of the translation initiation complex.[120] Upon stimulation of oocyte maturation, CPEB1 is phosphorylated by Aurora A/Eg2, PARN is removed, and GLD2 initiates polyadenylation. PABPC1L binds to the elongated poly(A) tails, breaking the Maskin-eIF4E association and establishes the "closed loop" through PABPC1L-eIF4G-eIF4E interactions. **(B)** Translation activation of the *Ringo/Spy* mRNAs is induced by removing PUM2 from the repression complex. Pumilio-binding elements (PBEs) at the 3′-UTR of *Ringo/Spy* mRNAs associate with their binding protein PUM2. PUM2 binds to PBE and represses its translation associated with DAZL and PABPC1L. *Xenopus* oocyte maturation is induced by progesterone; PUM2 dissociates from this complex and the DAZL-PABPC1L complex activates *Ringo/Spy* mRNAs translation.

detected in MII oocytes, zygotes, and 2-cell embryos, and is not detected at the 4-cell, 8-cell, and blastocyst stages[18] (Figure 3B). Uysal et al. produced a PABPC1L-specific antibody and profiled its spatial and temporal expression patterns during mouse MZT, including the PABPC1L homolog, PABPC1.[75] PABPC1L protein expression gradually decreased from GV oocytes to 2-cell embryos, while the PABPC1 protein expression gradually increased from GV oocytes to zygotes, and significantly decreased in 2-cell embryos (Figure 3B). PABPC1L and PABPC1 were distributed in both the cytoplasm and nucleus, while PABPC1 had a unique accumulation in the perinuclear space at the 2-cell stage.

PABPC1L is required for stabilizing maternal mRNAs and regulating their translational activation in mouse oocytes. Female *Pabpc1l* knockout mice are infertile because they fail to generate mature oocytes; however, *Pabpc1l*$^{-/-}$ males are fertile (Table 1 and Figure 1). *Pabpc1l*-deletion disrupts translational activation of maternal mRNAs during oocyte maturation, including *c-Mos*, *Ccnb1*, and *Dazl* mRNAs.[16] Additionally, *Pabpc1l*$^{-/-}$ oocytes display meiotic division and chromosome alignment defects during oocyte maturation in vitro.[16] Detailed analysis showed that compared to wild-type, *Pabpc1l*$^{-/-}$ oocytes were smaller in size, have peripheral germinal vesicles, and were loosely connected with cumulus cells. The surrounded nucleolus (SN) chromatin configuration and transcriptional silencing were disturbed in *Pabpc1l*$^{-/-}$ oocytes (Table 1 and Figure 1); however, microinjection of *Pabpc1l* mRNA into *Pabpc1l*$^{-/-}$ preantral follicle-enclosed oocytes reversed these defects and induced meiosis.[76] These findings indicated that PABPC1L plays an indispensable role during oocyte growth and determines the acquisition of meiotic competence.

In addition to disrupted oocyte growth and maturation, *Pabpc1l* null mice also exhibited deficient cumulus expansion and ovulation[16] (Table 1 and Figure 1). Because *Pabpc1l* is not expressed in the granulosa cells, it is most likely that oocyte-somatic communication is affected by *Pabpc1l*-deletion. To evaluate the interplay between the oocyte and somatic cells, a co-culture system of denuded oocytes (DOs) and oocytectomized COCs (OOXs) was established.[77] The experiment revealed that cumulus expansion was disrupted in both a WT-OOX/KO-oocyte and KO-OOX/WT-oocyte coculture system, indicating that oocytes and cumulus cells derived from *Pabpc1l*$^{-/-}$ mice were unable to transmit and accept the fundamental signals required for cumulus expansion. Further investigation suggested that MEK/ERK/p90RSK activation in response to LH and epidermal growth factor signaling was disturbed in granulosa cells with *Pabpc1l* deficiency.[77] Because PABPC1L is oocyte specific, the granulosa cell dysfunction in *Pabpc1l*$^{-/-}$ mice was attributed to the oocyte (Table 1 and Figure 1). Another study found that F-actin was reduced in late preantral follicles and was entirely absent

in COCs of *Pabpc1l*$^{-/-}$ mice, while E-cadherin was decreased in *Pabpc1l*$^{-/-}$ oocytes.[74] These analyses suggest that PABPC1L was required for oocyte-somatic communication through its maintenance of transzonal processes (TZPs) and gap junctions at the preantral stage of folliculogenesis[74] (Table 1 and Figure 1). The cross-talk between the oocytes and the granulosa cells persisted during folliculogenesis, and these phenotypes may be primarily derived from underlying defects in the extra oocyte-secreted factors that are directly caused by *Pabpc1l* deficiency in oocytes. Nevertheless, more detailed molecular mechanisms underlying PABPC1L contribution to these cellular processes require clarification.

NUCLEAR POLY(A) BINDING PROTEINS

General function of PABPNs

PABPN1 is the chief PABPN and highly conserved in all eukaryotes. The best-known function of PABPN1 is to stimulate polyadenylation, which has been broadly determined by biochemical analyses.[27,78,79] In eukaryotes, 3′-end processing of pre-mRNA transcripts is accomplished by two activities: endonuclease-triggered cleavage is followed by the addition of poly(A) tails to this upstream cleavage product conferred by poly(A) polymerase.[80] All of these are directed by the PAS in the substrate mRNA, which is recognized by CPSF.[81] Subsequently, CPSF-73 mediates pre-mRNA cleavage and recruits poly(A) polymerase to add adenosine residues to the mRNA 3′-end. Poly(A) polymerase by itself is rarely active because of its low binding ability of the RNA substrate.[81] Hence, PABPN1 binds the first 11 adenosines of the growing poly(A) tail and enhances the activity of the polymerase by directly tethering the enzyme to the mRNA and triggering processive polyadenylation.[27] Moreover, PABPN1 interacts with poly(A) polymerase through the coiled-coil domain in its N-terminal region, especially at leucine 136, and stimulates processive polyadenylation by poly(A) polymerase.[27]

Poly(A) tails with a defined length are typically added to mRNA, and PABPN1 functions to modulate the poly(A) tail length as a "molecular ruler".[82] PABPN1 covers the extending poly(A) tail to form a 21-nm spherical particle that allows the nascent poly(A) tail to fold back on itself, thereby keeping poly(A) polymerase in contact with CPSF.[78,82] The maximum particle size had a threshold length of ~ 250 adenosines in the PABPN1-poly(A) complex in vitro.[82] Upon reaching this threshold length, the spherical mRNA-protein complex can no longer accommodate extra PABPN1 molecules to facilitate CPSF-poly(A) polymerase interaction, which terminates polyadenylation.[78]

The nuclear accumulation of poly(A) mRNA was detected after depleting PABPN1 in primary muscle cells, suggesting that PABPN1 participates in poly(A) mRNA export from the nucleus.[83] Furthermore, PABPN1 is also found on the *Chironomus tentans* Balbiani ring (BR) mRNA ribonucleoprotein that is docked at the nuclear pore.[84] This suggests that PABPN1 is involved in mRNA export from the nucleus, although additional studies are required to clarify the explicit roles of PABPN1 during this process.

PABPN1 is also considered a director of alternative cleavage and polyadenylation (APA).[85] APA appears to be an important regulator of gene expression because it influences mRNA fate by altering RNA-binding protein and microRNA docking platform availability.[85,86] Genome-wide analysis showed that loss of PABPN1 resulted in a widespread enhancement of proximal cleavage sites (CS) that caused 3′-UTR shortening and thus, PABPN1 was defined as an APA suppressor.[85] Disordered APA was also observed in oculopharyngeal muscular dystrophy (OPMD) caused by a triplet repeat expansion in *PABPN1*.[85] Recent studies have uncovered a role for PABPN1 in RNA decay. A majority of long noncoding RNAs (lncRNAs) accumulated after PABPN1 deficiency, which facilitated lncRNA turnover in a polyadenylation-dependent manner in cooperation with exosome- and RNA helicase MTR4/SKIV2L2-mediated decay.[87] The relationship between PABPN1 and the RNA exosome is explained by poly(A) tail exosome targeting (PAXT) connection, which consists of the ZFC3H1 Zn-knuckle protein, which functions as a bridging protein associated with hMTR4 and PABPN1.[88] Thus, PAXT targets longer and extensively polyadenylated transcripts, such as the mature snoRNA host gene, *SNHG*.[88] PABPN1 also plays a role in promoting nuclear mRNA decay for guarantee proper mRNA "quality control". This pathway is established by PABPN1, the canonical PAPs, PAPα, and PAPγ, and the nuclear exosome subunits RRP6 and DIS3. RNA favored by this decay pathway depends on the PABPN1 and poly(A) polymerase-dependent growth of the poly(A) tail, and subsequent degradation via exosomes. Efficiently spliced endogenous mRNAs escape this decay pathway, supporting the mRNA quality control function of PABPN1.[89]

In summary, these studies underscore the well-documented role of PABPN1 in modulating poly(A) polymerase-dependent mRNA polyadenylation and expand our understanding of the functional repertoire of PABPN1, including nuclear RNA export, APA, and various RNA decay, as well as highlighting the need to characterize the full spectrum of PABPN1 functions.

The regulation of PABPNs

Although PABPN1 is ubiquitously expressed and contributes to multiple aspects of gene expression, *Pabpn1* mRNA is unstable, especially in skeletal muscle, suggesting that the control of *Pabpn1* expression is important for its function.[90] The *Pabpn1* 3′-UTR contains multiple *cis*-regulatory elements that are associated with *trans*-acting factors and coupled with spatial and temporal modulation of gene expression. Two conserved PAS are present in the *Pabpn1* 3′-UTR[90] and the distal PAS utilization defines the long isoform containing four putative AU-rich elements (AREs). Further analyses showed that the human antigen R (HuR) protein interacts with ARE4 and represses *Pabpn1* expression in mature muscle.[91]

The two isoforms of *Pabpn1* also trigger PABPN1 autoregulation. The distal PAS creates an unspliced pre-mRNA that includes intron 6. Increased PABPN1 protein is recruited to the poly(A) tail of the 3′-terminal intron retention isoform and promotes their clearance by the nuclear exosome, thus decreasing the protein levels of

endogenous PABPN1 and revealing homeostatic control of PABPN1 expression.[92]

Post-translational modification is a key factor in modulating PABPN1 activity. PABPN1 is involved in post-transcriptional processing that occurs both in the nucleus and cytoplasm; hence, regulating the nuclear-cytoplasmic distribution of PABPN1 is a prerequisite for maintaining these functions in an orderly manner. PABPN1 contains an evolutionarily conserved proline-tyrosine nuclear localization signal (PY-NLS) at its C-terminal, which is related to Kapβ2-mediated nuclear import in fission yeast; however, PY-NLS cargo is not responsible for PABPN1 nuclear localization in human cells.[93] Further studies found that human PABPN1 bears 13 asymmetrically dimethylated arginine residues at its C-terminus that bind the import receptor transportin and function as an NLS.[29] PRMT1, 3, and 6 methylate PABPN1 in vitro, which weakens the affinity of PABPN1 for transportin but favors RNA binding.[29,94] Thus, methylation plays a role in fine-tuning the competitive binding between transportin and RNA with PABPN1 to help coordinate the localization and function of PABPN1.

Recent studies have also revealed that PABPN1 is a novel target of ataxia-telangiectasia mutated (ATM). It has been reported that PABPN1 can be recruited at DNA double-strand breaks (DSBs) and be phosphorylated at Ser95 by ATM, which plays a vital role in the DNA DSB response.[95] Loss of PABPN1 increases the sensitivity of cells to DSB-inducing agents and lengthens DSB-induced G2/M cell-cycle arrest. Moreover, DSB repair is impeded by PABPN1 depletion or when its phosphorylation site is blocked. Further mechanistic analyses indicated that PABPN1 is recruited to DSB sites and phosphorylated on Ser95, which effectively resects DNA-ends in both non-homologous end-joining and homologous recombination repair.[95]

PABPNs in gametogenesis and early embryogenesis

The functions of PABPN1 were characterized through biochemical work in cell lines; however, the physiological significance of PABPN1 during gametogenesis and embryogenesis is still largely unknown. A study on *Drosophila melanogaster* provided insight into the potential roles of PABPN1 in oocytes and early embryos.[96] *Drosophila* PABPN1 ortholog PABP2 has been described to be present in the cytosol of oocytes and early embryos.[97] Mutations in *Pabp2* resulted in poly(A) tail extension for definite mRNAs regulated via cytoplasmic polyadenylation, therefore increasing the protein levels encoded by these transcripts. This cytoplasmic role of PABP2 is essential for development since *Pabp2*-depleted embryos showed early developmental arrest.[96] PABP2 is well-known for its role in nuclear polyadenylation but has a cytoplasmic function during early development.[96,97] More experimental evidence is required to clarify the mechanism by which *Drosophila* PABPN1 trims poly(A) tails of cytosolic transcripts; however, these findings address the cytoplasmic function of PABPN1 during early development, which differs significantly from somatic cells.

An embryonic poly(A)-binding protein 2 (ePABP2; ortholog of mammalian PABPN1L) was identified in *Xenopus*, mouse, and the human genome, which had a similar structure to the PABPNs.[98,99] The

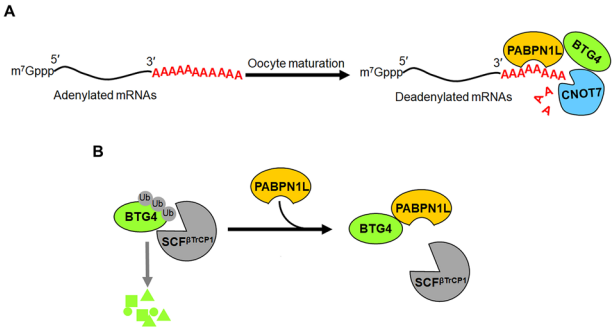

FIGURE 5 PABPN1L functions during mouse MZT. **(A)** During oocyte maturation and fertilization, PABPN1L tethers BTG4 and CCR4-NOT deadenylase to the poly(A) tails of maternal transcripts to facilitate cytoplasmic mRNA decay. **(B)** PABPN1L protects BTG4 from polyubiquitination by SCF$^{\beta TrCP1}$ and degradation.

Pabpn1l expression pattern was also characterized in oocytes and early embryos but differed from that of its ubiquitously expressed homolog, PABPN1.[19] PABPN1L acts as a poly(A) adaptor of the mammalian MZT licensing factor B-cell translocation gene-4 (BTG4), which mediates maternal mRNA clearance.[19] BTG4 recruits the CNOT7 catalytic subunit of CCR4-NOT deadenylase to maternal mRNAs and triggers their degradation.[100–102] However, BTG4 *per se* does not contain an RNA-binding domain. The expression window of PABPN1L spatiotemporally overlapped with the expression pattern of BTG4 and the MZT process, which gradually accumulates after meiotic resumption and quickly decreases after fertilization (Figure 3B). Both *Pabpn1l* and *Btg4* knockout mice showed similar phenotypes in that female null mice were sterile owing to the derived embryos arrested at the 1- to 2-cell stage (Table 1 and Figure 1). These results provided genetic evidence that PABPN1L is involved in the BTG4-mediated biological process. Furthermore, deletion of *Pabpn1l* impaired deadenylation and degradation of a subset of BTG4-targeted maternal mRNAs. Mechanistic investigation determined that PABPN1L bound to the 3′-poly(A) tail of maternal transcripts recruited BTG4 and CCR4-NOT deadenylase and mediated their cytoplasmic decay during MZT (Figure 5A and Table 1). Moreover, BTG4 failed to accumulate in *Pabpn1l*$^{-/-}$oocytes. Analyses revealed that PABPN1L stabilized the BTG4 protein by preventing it from being polyubiquitinated by SCF$^{\beta TrCP1}$ and degraded[19] (Figure 5B and Table 1). This study provided new mechanistic insight into nuclear poly(A)-binding proteins and highlights the physiological importance of PABPN1L during MZT.

The appealing function of nuclear PABPs in regulating cytoplasmic mRNA decay during early embryonic development underscores their basic working principle in somatic cells, and whether this turning of PABPNs influences mRNA translation or decay has not yet been recognized. Whether developmental stage-specific regulators alter the cytoplasmic availability of PABPNs during MZT and determine the roles of loaded PABPNs in modulating the vulnerability of targeted mRNA towards either poly(A) polymerase or deadenylase remains unclear. Further investigations into the mechanism underlying the regulation of PABPs in multipronged functions during gene expression are urgently needed.

PERSPECTIVES

Germ cell differentiation depends on spatially and temporally regulated gene expression, which is generated by orchestrated waves of development stage-coupled transcriptional activities. Transcriptional silencing occurs in the late steps of gametogenesis, which is intrinsically considered the reprogramming process, reflecting the dormant state of the chromatin until zygotic transcription recovery.[103,104] Indeed, some factors associated with organizing the chromatin architecture contribute to transcriptional silencing.[105–108] To deal with incapacitated transcription, specialized post-transcriptional mechanisms are generated to ensure that all transcripts meet their destinated fate, especially for genes that are transcribed earlier but translationally repressed until needed. To the best of our knowledge, this process relies heavily on the regulation of their poly(A) tails.[109] The prevailing modified TAIL-seq partially revealed the processes associated with poly(A)-tail profiling during gametogenesis and early embryogenesis: (1) coupling of tail lengths and translational efficiencies was observed in mouse GV oocytes, and zebrafish, early-stage frog embryos, yet absent in non-embryonic samples[110,111]; (2) maternal transcripts are detected with APA isoform-specific poly(A) tails in mouse GV oocytes[110]; and (3) widespread non-adenosine residues are found within maternal mRNA poly(A) tails. Basically, PABPs are organized on poly(A) tails; they determine mRNA stability and translation. These emerging findings advance our understanding of the functions of PABPs, including PABPN1, which serves as the master in determining APA,[85] and the non-poly(A) sequence binding affinity of PABPC1 and PABPN1, indicating the potential regulation of PABPs in germ cells and early embryos.[35,112] It is highly important to investigate the global dynamics of poly(A) tails during gametogenesis and early embryogenesis, as well as the effects of PABPs, such as those on mRNA storage and translational activation, during the rapid developmental switch, in particular, with transcriptional shutdown. The regulation of germ cell-specific PABP expression merits further clarification because of the importance of the physiological processes in which PABP functions. For instance, both *Pabpc1l* and *Pabpn1l* are oocyte-specific PABPs in *Xenopus* and mice, and female mice null mutation at either loci are infertile, however, owing to absolutely different phenotype. It was reported that PABPC1L plays a role in oogenesis[74] while in contrast, PABPN1L is crucial for MZT[19] (Figure 1). Their high mRNA levels in oocytes suggest that post-transcriptional regulation determines developmental stage-coupled protein accumulation, which is a prerequisite for physiological importance. However, the underlying mechanism of this role remains under-investigated.

With the appearance of germ cell-predominant PABPs, the functions of orthologous canonical PABPs are largely ignored. Given the significance of PABP function demonstrated by cell biological experiments, the physiological mechanism of canonical PABPs should be characterized. As described, PABPN1L is a homolog of the ubiquitously expressed PABPN1, and mouse oocyte transcriptomics revealed that the level of *Pabpn1* is the second-highest among detected PABPs.[19] It is still an open question as to whether there is coordination between PABPN1 and PABPN1L in common processes or whether they are involved in different biological processes. Thus, very little is known about the function of PABPN1 during oogenesis. It is noteworthy that gradual accumulation of *Pabpn1* during preimplantation development was observed, compared to a nearly undetectable level of *Pabpn1l*.[19] The transgenic mouse expressing the alanine-expanded PABPN1 under a ubiquitous promoter displays muscle pathology, followed by developmental retardation and a shortened lifespan without preimplantation developmental defects.[113] Clearly, PABPN1-mediated APA is not as important as described in muscle cells. Further studies are needed to define the intrinsic function of PABPN1 during early embryogenesis. According to well-established classification, two distinct groups of PABPs are defined by their steady-state localization: PABPCs and PABPNs localize to the cytoplasm and the nucleus, respectively.[114] Yet, PABPN1 was observed in the cytosol in electron micrographs[115] which challenges this classification. PABPC1 was also detected in the nucleus in yeast[116] and mammalian cells.[117] Thus, PABPN1 and PABPC1 have both been described as shuttling proteins rather than having stable localization. To date, a limited number of functional studies have been conducted to support this shuttling behavior. Given the cytosolic function of PABPN1 in *Drosophila* early embryos, we believe that early embryos provide an ideal model for investigating the potential cytoplasmic function of PABPN1 because its well-known nuclear function does not seem to be prevalent. With the lack of a clear boundary between the localization of PABPN1 and PABPC1, it is unclear how the multiple PABPs coordinate during mRNA post-transcriptional processing. Two plausible models are proposed: (1) poly(A)-bound PABPN1 is replaced by PABPC1 in the cytoplasm and vice versa; and (2) PABPC1 and PABPN1 combine with the same mRNA poly(A) tail. However, the mechanisms of these models should be further evaluated for understanding the cooperation of PABPs in regulating gene expression.

The expression of germ cell-specific PABPs and their physiological importance for germ cell and fertilized embryo developmental competence make them good candidates for medical diagnosis of human infertility. Infertility is a global public health issue estimated to affect between 8% and 12% of reproductive-aged couples worldwide.[118] A better understanding of the germ cell-specific physiological functions of PABPs could enhance our diagnostic capabilities for human infertility and may pave the way for advancing new therapies in the clinic.

CONCLUSIONS

In this review, we have highlighted the importance of canonical and novel poly(A)-binding proteins in post-transcriptional regulation during and after gametogenesis, and early embryogenesis. It is clear that PABPs play an indispensable role in gametogenesis and early embryogenesis, as displayed by the unconventional roles of PABPs in regulating gene expression as well as by novel PABP family members that are specifically present in germ cells. The correct accumulation of RNAs during the developmental process is balanced by tethering RNAs to the translation apparatus and triggering their spatiotemporal degradation. PABPs act as scaffolds for both poly(A) tail and multifunctional protein

partners that control RNA fate, which is pivotal to generating developmentally competent gametes and embryos. Discovering additional PABP members and understanding the molecular mechanism of these PABPs alone or in combination will not only advance our knowledge of post-transcriptional regulatory networks but also provide mechanistic insights into gametogenesis and early embryogenesis, as well as provide potential options for reproductive therapy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

No experimental data were generated for this review manuscript.

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