—Review—

Potential roles of the poly(A)-binding proteins in translational regulation during spermatogenesis

Saffet OZTURK[1] and Fatma UYSAL[1]

[1)]*Department of Histology and Embryology, Akdeniz University, School of Medicine, Antalya, Turkey*

Abstract. Spermatogenesis is briefly defined as the production of mature spermatozoa from spermatogonial stem cells at the end of a strictly regulated process. It is well known that, to a large extent, transcriptional activity ceases at mid-spermiogenesis. Several mRNAs transcribed during early stages of spermatogenesis are stored as ribonucleoproteins (RNPs). During the later stages, translational control of these mRNAs is mainly carried out in a time dependent-manner by poly(A)-binding proteins (PABPs) in cooperation with other RNA-binding proteins and translation-related factors. Conserved PABPs specifically bind to poly(A) tails at the 3′ ends of mRNAs to regulate their translational activity in spermatogenic cells. Studies in this field have revealed that PABPs, particularly poly(A)-binding protein cytoplasmic 1 (Pabpc1), Pabpc2, and the embryonic poly(A)-binding protein (Epab), play roles in the translational regulation of mRNAs required at later stages of spermatogenesis. In this review article, we evaluated the spatial and temporal expression patterns and potential functions of these PABPs in spermatogenic cells during spermatogenesis. The probable relationship between alterations in PABP expression and the development of male infertility is also reviewed.

Key words: Male infertility, Poly(A)-binding protein (PABP), Spermatogenesis, Translational control

Introduction

The development of male germ cells begins with the appearance of a group of primordial germ cells (PGCs) on the yolk sac wall. The PGCs arise from the epiblast layer and undergo many mitotic divisions during their journey from the yolk sac, along the dorsal mesentery, to the genital ridge. In the primitive gonads, PGCs complete their mitotic divisions and then differentiate into gonocytes. Similarly, gonocytes experience mitotic divisions and eventually differentiate into spermatogonial stem cells [1]. Briefly, spermatogenesis is defined as the differentiation of diploid spermatogonial stem cells into haploid spermatozoa at the end of strictly regulated consecutive phases. Three main phases can be distinguished during spermatogenesis: spermatocytogenesis, meiosis, and spermiogenesis [2]. During spermatocytogenesis, two different types of type A spermatogonial stem cells exist: dark and pale, both of which undergo many mitotic divisions for both self-renewal and differentiation. In the process of differentiation, some of the pale type A spermatogonial cells generate type B spermatogonia. After that, type B spermatogonia, having cytoplasmic bridges, enter the first round of meiotic division, after which they are termed primary spermatocytes. In the meiotic phase, primary spermatocytes complete the first meiotic division to yield secondary spermatocytes. These, in turn, give rise to haploid round spermatids after completing the second meiotic division. Spermiogenesis, the third phase of spermatogenesis, is the transformation of postmeiotic round spermatids into spermatozoa following the completion of the Golgi, acrosome, cap, and maturation phases. During spermiogenesis, prominent morphological changes occur: the formation of the acrosome and the flagellum, nuclear condensation and elongation, elimination of the cytoplasm, and reorganization of organelles, involving the formation of the mitochondrial sheath at the middle part of the sperm tail [3, 4].

Spermatogenesis [5] has its similarities and differences with oogenesis [6]. In both processes, meiotic division occurs, at the end of which haploid gametes are formed. On the other hand, transcriptional activity ceases at the beginning of nuclear/cytoplasmic maturation in oocytes, while it ceases at mid-spermiogenesis in testes. Furthermore, spermatogenesis and oogenesis take place in different reproductive organs and under the control of distinct hormones. In addition, oogenesis is initiated during fetal life and spermatogenic activity at the onset of puberty. Finally, at the end of spermatogenesis, four spermatozoa are formed, while, at the end of oogenesis, only one mature oocyte and two polar bodies are generated. It is important to note that spermatogenic cells experience marked morphological changes during the spermiogenesis stage. As a result, there are many molecular biological differences between spermatogenesis and oogenesis that have been characterized to date.

The three phases of spermatogenesis are largely regulated by stage-specific gene expression [7]. Precursor mRNA processing is a well-known and important step in the control of gene expression, and it constitutes a part of posttranscriptional regulation. During this process, newly transcribed mRNAs undergo particular post-

transcriptional modifications at either the 5′ or the 3′ end, such as capping, polyadenylation, and excision of introns (splicing) [8, 9]. Capping is the addition of 7-methylguanosine (m⁷G) to the 5′ end, so that the 5′ end of newly transcribed mRNAs is protected from potential nuclease attacks. Poly(A) tails are added to the 3′ end of newly transcribed mRNAs in the nucleus under the control of *cis*-acting sequences and *trans*-acting factors [9]. The *cis*-sequences (AAUAAA or AUUAAA, known as poly(A) addition signals), localized 15–30 nucleotides upstream of the mRNA cleavage region at the 3′ end, specifically interact with *trans*-acting factors such as the cleavage and polyadenylation specificity factor (CPSF) [9]. In addition, poly(A) polymerase in the nucleus carries out the addition of approximately 150–250 adenosine residues to the 3′ end of mRNA [10]. It is important to note that, as several germ cell genes like testicular actin-capping protein alpha subunit (*Gsg3*; also known as *Capza3*) do not have a poly(A) addition signal, their transcripts do not experience polyadenylation [11]. In the process of intron excision, a spliceosome complex primarily plays the role of removing intervening sequences (i.e., introns) from precursor mRNAs [12]. These modifications are carried out in the nucleus and have critical roles in the export of mRNAs into the cytoplasm, translational regulation, and mRNA decay [13–15]. Following transport of mature mRNAs into the cytoplasm, cytoplasmic poly(A)-binding proteins (PABPs) specifically associate with the poly(A) tails in place of nuclear PABPs, which were bound to the poly(A) tails in the nucleus [16, 17].

There are two different translation mechanisms described in eukaryotic cells: cap-dependent and cap-independent. In the cap-dependent mechanism, a large translation complex, consisting of eukaryotic translation initiation factor (eIF) 4F (which is composed of eIF4A, eIF4E, and eIF4G), eIF4B, PABPs, and the remaining translation-related factors (eIF1, eIF1A, eIF2, eIF3, and eIF5), is initially formed [18]. eIF4G creates a bridge between eIF4E and PABPs, so that it stabilizes the interactions among translation factors, and a 'closed loop' structure is created thereby [19]. Its basic function is to protect 3′ and 5′ ends of mRNAs from potential nuclease attacks and to induce translation activity [19]. Finally, the ribosomal complex, which is composed of large and small subunits, can translate the mature mRNAs in cooperation with the translation-initiation factors based on the requirements of the cell. It is important to note that most eukaryotic mRNAs undergo cap-dependent translation, which means that most mRNAs undergo translational control dependent on posttranscriptional modifications. In the cap-independent translation mechanism, special structures, known as hairpins and pseudoknots [20], as well as sequences such as internal ribosome entry sites and upstream open reading frames [21] at the 3′ or 5′ UTR regions of the mRNA help initiating the translation of the target mRNA.

During spermatogenesis, transcriptional activity is suppressed at mid-spermiogenesis and onward [22, 23]. Therefore, early haploid spermatids, as well as spermatogonia and spermatocytes, transcribe a large number of mRNAs with long poly(A) tails (~150 nucleotides), which are then stored as translationally inactive ribonucleoprotein particles (mRNPs) [24, 25]. Sperm-specific nuclear protein genes encoding protamines (Prm) and transition proteins (Tp) are the best-known examples of translational control during spermatogenesis. The *Prm* and *Tp* genes are highly expressed and stored as mRNPs in round spermatids during the early stages of spermiogenesis [26–28].

Histones in nucleosomes are replaced by transition proteins in elongating/elongated spermatids and then by protamines throughout later stages of spermiogenesis; this results in transcriptional silencing [27]. The translationally repressed *Prm* and *Tp* mRNAs in round spermatids have poly(A) tails of approximately 180 nucleotides. When *Prm* and *Tp* mRNAs become translationally active in elongating/elongated spermatids, their poly(A) tails decrease to approximately 30 nucleotides in order to stimulate their interaction with ribosomes and initiate translation [25, 27]. The translational activation of the mRNAs in later spermiogenesis is compatible with poly(A) tail shortening [27], which is different from the process in oocytes, in which poly(A) tail lengthening occurs [29]. The proteins required at later stages of spermatogenesis are translated following poly(A) tail shortening from previously repressed mRNAs [27].

Structure of PABPs

There are two main groups of PABPs based on structural, functional, and intracellular localization: cytoplasmic and nuclear PABPs. Cytoplasmic PABPs, including Pabpc1, Epab, and Pabpc2 (known as PABPC3 in humans), are composed of three major domains: four RNA recognition motifs (RRMs) at the N-terminus, a proline-rich region, and a PABC domain at the C-terminus [30, 31]. The RRM domains are capable of binding specifically to poly(A) chains that consist of 25–27 adenosines. While RRMs 1–2 and RRMs 3–4 interact strongly with adenosine residues, RRMs 2–3 associate weakly with long stretches of adenosines. It is important to note that RRMs 1–2 bind exclusively to sequences that include adenosines, whereas RRMs 3–4 can also bind to sequences that include other nucleotides, such as AU-rich sequences [32, 33]. The unstructured and globular PABC domain enables it to slightly induce translational activity when tethered to a reporter mRNA [34], and it also participates in protein–protein interactions such as the binding of PABC to PABP-interacting motif (PAM) found in PABP partners [35]. The proline-rich region contributes to PABP self-interaction and participates in concomitantly binding PABPs to poly(A) tails [36]. Overall, PABPs can bind with mRNPs and polysomal mRNAs, suggesting that they likely participate in the storage, decay, and translational regulation of mRNAs in spermatogenic cells during spermatogenesis [37, 38]. The structural and functional features of PABPs have been extensively evaluated in our previously published review article [39].

PABPs establish a direct interaction with PABP-interacting proteins (PAIPs), including PAIP1, PAIP2A, and PAIP2B. PAIP1 is capable of inducing translational activity by simultaneously associating with eIF3, eIF4A, and PABPs [40, 41]. By contrast, PAIP2 decreases the affinity of PABPs for binding to poly(A) tails, because PAIP2 competes with PAIP1 or eIF4G for binding to PABP; therefore, PAIP2 leads to translational inhibition both *in vitro* or *in vivo* [42, 43]. The two different PAMs in PAIP2 can bind to the RRMs and the PABC domain of PABPs to repress their action [44]. As PAIP2 specifically suppresses the translation of mRNAs with poly(A) tails, it does not inhibit the translation of mRNAs that are translated in the cap-independent manner. It is important to note that PAIP2 also competes with PAIP1 for binding to PABPs, which prevents PABPs from promoting translational activity [43]. Male mice lacking the

Paip2 isoform *Paip2a* or both *Paip2a* and *Paip2b* isoforms are infertile because mRNAs required during male germ cell development are not properly translated in elongating spermatids [28]. In *Paip2a*/*Paip2b* double-knockout mice, PABP expression is aberrantly increased, and it may impair the establishment of the bridge linking eIF4E and the cap structure at the 5′ end of mRNAs [28]. This finding suggests that optimal PABP levels are necessary for proper translational activity during late spermiogenesis. In a study by Delbes *et al.* (2012), it was found that, throughout spermiogenesis in mice, Paip2a helps to control important proteins, notably Eif4g1, A kinase anchoring protein (Akap4), and hexokinase-1 (Hk1) [45].

PABPC1 during Spermatogenesis

PABPC1, also known as PABP1 and PAB in *Saccharomyces cerevisiae*, was first cloned using human melanoma cells and described by Grange *et al.* (1987) [46]. In the same year, Sachs *et al.* (1987) revealed that the lack of the *Pab1* gene in yeasts is lethal [47]. Among PABPs, the ubiquitously expressed PABPC1 is also known as the somatic PABP, and it has been extensively studied in the context of translational regulation of mRNAs in eukaryotic cells [48, 49].

Pabpc1 mRNA expression exhibits dynamic changes in spermatogenic cells during spermatogenesis in mice: it increases at the meiotic stage, reaches peak levels in the early postmeiotic stages, and decreases down to being undetectable by the end of spermatogenesis [37]. Consistently, the levels of Pabpc1 reach maximum levels in postmeiotic round spermatids in the mouse testes (Fig. 1A) [37]. Although the levels of *Pabpc1* mRNA are low in elongating spermatids [50], the presence of the Pabpc1 protein indicates that its mRNA stability is maintained through the end of the spermatogenesis process (Fig. 1A) [37].

EPAB during Spermatogenesis

Epab, also known as Epabp and Pabpc1-like, includes almost the same structural domains that exist in Pabpc1; however, Epab exhibits prominent differences in the amino acid content of either the RRM3 domain or the proline-rich region [51]. Epab primarily functions in preventing deadenylation of poly(A) tails, inducing cytoplasmic polyadenylation, and enhancing translational activity [51, 52]. Epab binds to the AU-rich element (UUAUUUAUU) at the 3′ end of mRNA, as well as to poly(A) tails, and then associates with polysomes to activate translation [52].

Several studies have revealed that the *EPAB* gene is highly expressed in *Xenopus* [51, 52], mouse [50, 53], and human [54, 55] germ cells, as well as in early embryos before embryonic genome activation (EGA), and it is likely replaced with PABPC1 following EGA. In RNA *in situ* analyses of mouse testes, *Epab* mRNA expression was detected at different levels only in spermatogenic cells and was not present in somatic cells [50]. Epab protein expression progressively declines from spermatogonia to spermatocytes, and the marked increase in Epab expression in round spermatids gradually decreases toward sperm cells in mice (Fig. 1B; unpublished data). Although *Epab* exhibits specific expression patterns in male germline cells in mice, *Epab* knockout male mice were found to be fertile [55]. Furthermore, the *Epab* knockout male mice were phenotypically

normal, and there were no differences in the body weight and sperm parameters, such as count, morphology, motility, and apoptosis index, when compared to either wild-type mice or their heterozygous knockout counterparts [56]. However, in the testes of *Epab* knockout male mice, compared to wild-type mice, the mRNA expression of *Msy2*, an RNA-binding protein specifically generated in germ cells, was significantly higher than that of other RNA-binding protein genes (*Pum2*, *Dazl*, *Pabpc1*, and *Paip2*) [56].

On the other hand, *Epab* knockout female mice are infertile due to abnormalities in oocyte maturation, folliculogenesis, cumulus expansion, and ovulation [55]. In a more detailed analysis, Lowther *et al.* (2015) reported that Epab is implicated in chromatin organization, transcriptional silence, and meiotic competence throughout the early stages of oogenesis in mice [57]. In addition, the phosphorylation levels of Mek1/2, Erk1/2, p90 ribosomal S6 kinase, and epidermal growth factor (EGF) receptor in *Epab* knockout mouse granulosa cells markedly decrease after treatment with either luteinizing hormone or EGF [58]. A recently published study revealed that Epab also contributes to oocyte-somatic communication by providing maintenance of transzonal projections and gap junctions, which are established between oocytes and granulosa cells in mouse preantral stage follicles [59].

A limited number of studies have been performed to determine the potential roles of the *EPAB* gene in human testes. Guzeloglu-Kayisli *et al.* (2008) first characterized the presence of *EPAB* mRNA in human testes and ovaries in addition to in somatic tissues, including the placenta, liver, kidneys, pancreas, spleen, and thymus [54]. The same study detected high levels of *PABPC1* mRNA expression in testis tissues [54]. In the same year, Sakugawa *et al.* (2008) published the results of a similarly designed study, showing that *EPAB* mRNA is expressed in human testis tissues, as well as in other somatic tissues, such as the lungs, liver, kidneys, pancreas, spleen, thymus, and colon [60]. However, the structural domains of EPAB are differently identified in the two studies. EPAB was defined as having four RNA recognition motifs and one C-terminal PABP domain (also known as the PABC domain) in the former study [54], and only three RRM motifs and no C-terminal PABP domain were described in the latter study [60]. As EPAB belongs to the cytoplasmic family of PABPs and plays a role in translational regulation, it is more likely that EPAB possesses the PABP domain to interact with the other translation-related proteins. The structural features and domains of the EPAB protein should be precisely addressed in future research.

In another study, Guzeloglu-Kayisli *et al.* (2014) examined the expressional control of the *EPAB* gene in the human somatic tissues [61]. Strikingly, they found that the *EPAB* mRNA undergoes posttranscriptional control in somatic tissues as well as in 8-cell and blastocyst stage embryos [61]. In these cells, an alternative splice form of the *EPAB* mRNA is transcribed because it does not have a functional poly(A)-binding domain due to being formed prematurely because of a stop codon on exon 8 [61]. It remains unknown whether the same mechanism takes place in spermatogenic cells during spermatogenesis in humans. Ozturk *et al.* (2016) revealed that *EPAB* is expressed in the spermatocyte and round spermatid fractions isolated from patients with non-obstructive azoospermia [62]. It is important to note that the spatiotemporal expression patterns and potential roles of EPAB should be ascertained in human spermatogenic cells
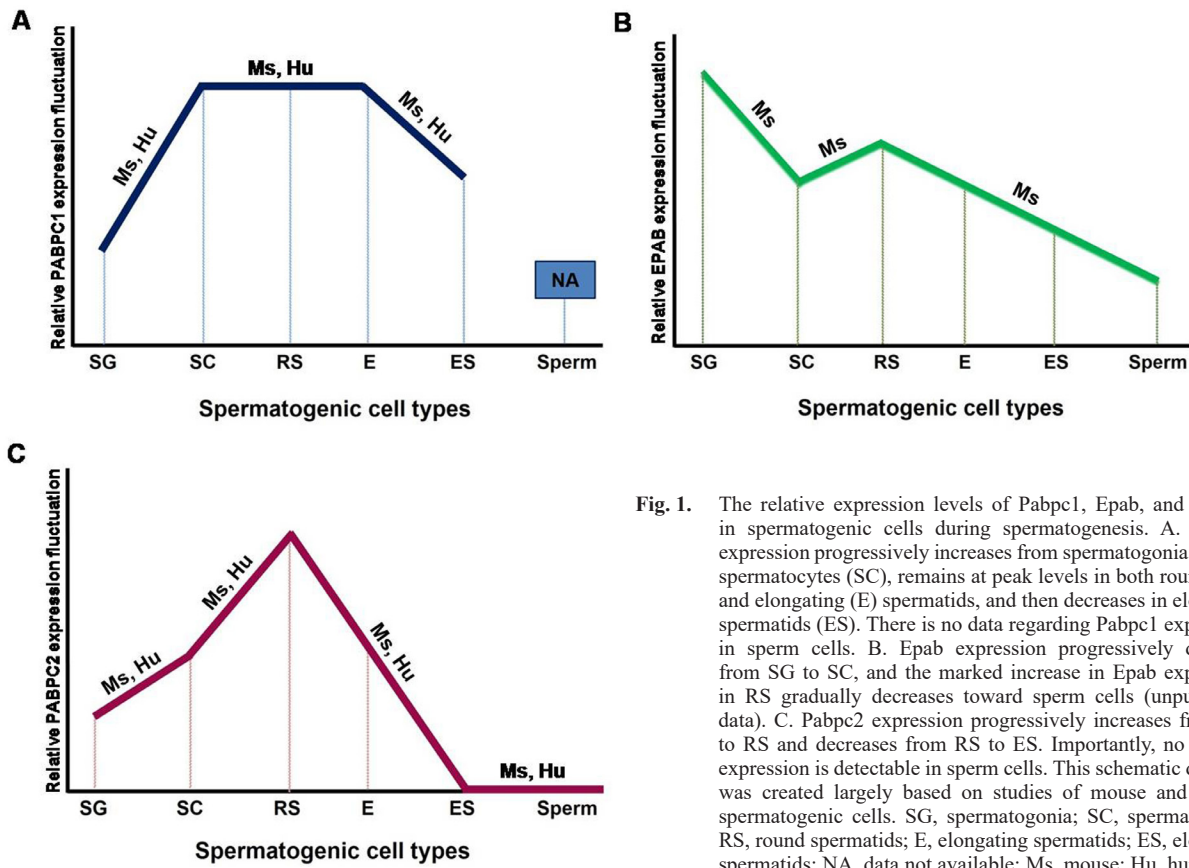
Fig. 1. The relative expression levels of Pabpc1, Epab, and Pabpc2 in spermatogenic cells during spermatogenesis. A. Pabpc1 expression progressively increases from spermatogonia (SG) to spermatocytes (SC), remains at peak levels in both round (RS) and elongating (E) spermatids, and then decreases in elongated spermatids (ES). There is no data regarding Pabpc1 expression in sperm cells. B. Epab expression progressively declines from SG to SC, and the marked increase in Epab expression in RS gradually decreases toward sperm cells (unpublished data). C. Pabpc2 expression progressively increases from SG to RS and decreases from RS to ES. Importantly, no Pabpc2 expression is detectable in sperm cells. This schematic diagram was created largely based on studies of mouse and human spermatogenic cells. SG, spermatogonia; SC, spermatocytes; RS, round spermatids; E, elongating spermatids; ES, elongated spermatids; NA, data not available; Ms, mouse; Hu, human.
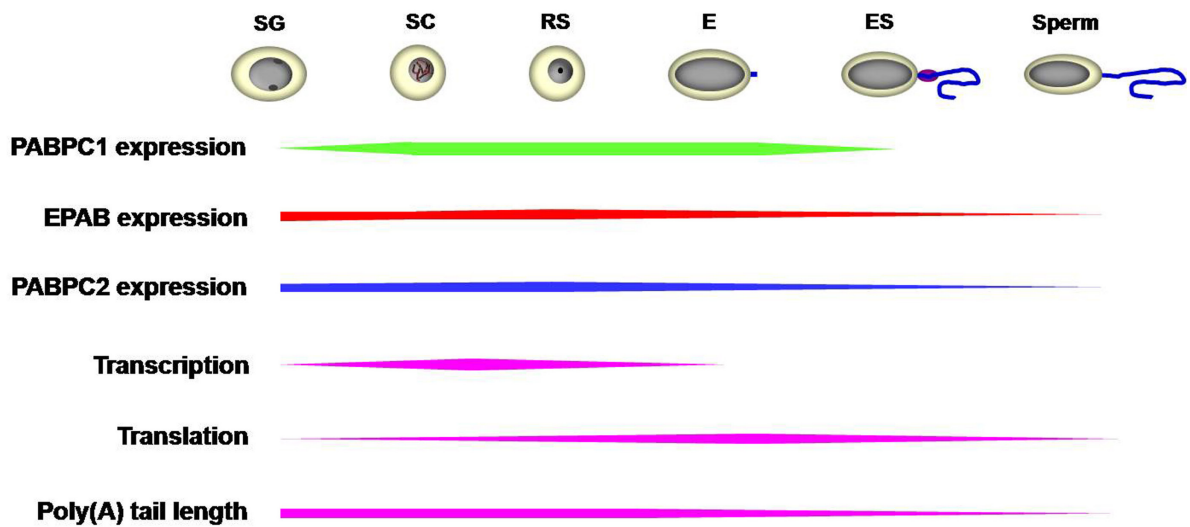

Fig. 2. Schematic diagram of Pabpc1, Epab, and Pabpc2 expression and cellular dynamics in spermatogenic cells during spermatogenesis. Pabpc1, Epab, and Pabpc2 exhibit expressional fluctuations from spermatogonia to sperm cells. The thicknesses of the lines in the figure represents the expression levels of the PABPs. Although transcriptional activity ceases to a large extent in elongating spermatids at mid-spermiogenesis, translational activity can be observed during spermatogenesis as well, but at different levels. The poly(A) tails of the stored mRNAs are shortened once they undergo translation. Each parameter is depicted in a different color. SG, spermatogonia; SC, spermatocytes; RS, round spermatids; E, elongating spermatids; ES, elongated spermatids.

during spermatogenesis.

On the other hand, several studies have evaluated the function of EPAB during translation. Studies that aimed to determine the potential relationship of EPAB with other translation-associated factors have resulted in the discovery of two complexes, the polyadenylation-dependent and the polyadenylation-independent complex. In the polyadenylation-dependent complex, EPAB interacts with a complex composed of cytoplasmic polyadenylation element binding protein 1 (CPEB1), CPSF, symplekin, and GLD2, a cytoplasmic poly(A) polymerase, to regulate the translational activity of the target mRNA [63]. In the polyadenylation-independent complex, there is an association between EPAB, deleted in azoospermia-like (DAZL), and pumilio 2 (PUM2), and this complex plays an important role in the translational activation of mRNAs without poly(A) tails [64]. We believe that new, more detailed studies are required to determine the potential effects of EPAB on mRNAs that undergo polyadenylation-dependent or -independent mechanisms.

PABPC2/PABPC3 during Spermatogenesis

The intronless *Pabpc2* gene (also known as *tPABP* and *PABPC3* in humans) is solely expressed in spermatogenic cells [65, 66]. *Pabpc2* is a paralogous gene that likely derives from a reverse transcriptase copy of a processed *Pabpc1* mRNA, which was then inserted into the genome and is therefore considered a retroposon [67].

The human testis-specific *PABP* gene *PABPC3*, first characterized by Feral *et al.* (2001), encodes a 631-amino acid protein (with a molecular weight of 70.1 kDa) that has a 92.5% similarity with the PABPC1 protein [68]. Northern blot analysis on several types of human somatic and gonadal tissues revealed that only human testes express two RNA isoforms (2.1 kb and 2.5 kb) of the *PABPC3* gene and that *PABPC1* mRNA (3.2 kb) is transcribed in all tested tissues [68]. Interestingly, among spermatogenic cells, *PABPC3* mRNA has been detected only in round spermatids, whereas *PABPC1* is transcribed either in pachytene spermatocytes or round spermatids in human adult testes [68]. It has been reported that PABPC3 is able to bind poly(A) tails with a lower affinity than PABPC1 [68]. The *PABPC3* gene does not include any introns as is observed in the *Pabpc2* gene in mice. In addition, the sequences located upstream of the transcription start region of *PABPC3* most likely provide promoter activity and have a tissue-specific expression [68].

On the other hand, Pabpc2 is exclusively expressed in pachytene spermatocytes and round spermatids in mice. Pabpc1 is, however, still present in elongating spermatids, as well as in previous stages of spermatogenic cell development (Figs. 1A and C) [66]. Both Pabpc1 and Pabpc2 potentially play a role *in vitro* in binding to the poly(A) tails of mRNAs to stimulate the translation of a reporter mRNA and interact with each other, as well as with various translation-related factors including Eif4g1, Paip1, Paip2, and Piwi-like protein 1 (Piwil1; previously known as Miwi) [66]. Although Pabpc2 does not have an active role in translating polyribosomes of spermatogenic cells, like Piwil1, it accumulates in the chromatoid body of round spermatids. However, Pabpc1 is in intensive contact with polyribosomes and mRNPs [66]. Taken together, while Pabpc2 is exclusively expressed in mouse spermatocytes and round spermatids (Fig. 1C) [66], the human PABPC3 is expressed only in the round spermatids [68]. The

species-specific expressional differences of the Pabpc2 and PABPC3 proteins in spermatogenic cells should be further evaluated in future studies. In addition, the finding by Paynton (1998) that *Pabpc2* mRNA is present at very low levels in mouse oocytes and early embryos should also be regarded [69].

Strikingly, mutant mice lacking *Pabpc2* that were generated by Kashiwabara *et al.* (2016) exhibited normal fertility, spermatogenesis, and sperm migration [70]. Additionally, haploid-specific mRNAs were correctly transcribed in round spermatids and translated in elongating spermatids [70]. These findings suggested that Pabpc2 seems to be a functionally redundant PABP that is not needed in the translational regulation at later stages of spermatogenesis. Most likely, the lack of the Pabpc2 protein in spermatogenic cells might be compensated by other known or still undefined RNA-binding proteins.

There are similarities and differences in the expressional distribution of Pabpc1, Epab, and Pabpc2 in spermatogenic cells during spermatogenesis (Figs. 1A, B, C). Pabpc1 expression progressively increases from spermatogonia to spermatocytes, remains at peak levels in round and elongating spermatids, and gradually declines toward elongated spermatids [66]. On the other hand, Epab expression is highest in spermatogonial cells and gradually decreases toward spermatocytes, and the slightly increased Epab expression in round spermatids gradually declines toward sperm cells (unpublished data). Pabpc2 expression progressively increases from spermatogonia to round spermatids [66]. Interestingly, it then gradually decreases from round to elongating spermatids, and eventually reaches undetectable levels in both elongated spermatids and sperm cells [66].

In conclusion, to summarize all information related to the PABPs and cellular events partially or largely controlled by these proteins, we created a schematic diagram that shows the expressional distribution of Pabpc1, Epab, and Pabpc2, their transcriptional and translational activities, and poly(A) tail length in spermatogenic cells during spermatogenesis (Fig. 2).

PABPs in Male Infertility

Male factor infertility affects 40–50% of infertile couples and is observed in approximately 15% of couples worldwide [71, 72]. Azoospermia is the absence of sperm in the ejaculate and is one of the most common male infertility factors. There are two types of azoospermia: obstructive azoospermia, which originates from an obstruction in the male genital tract, and non-obstructive azoospermia, which is caused by failure in producing sperm cells [73].

There is a limited number of studies that have analyzed whether PABPs have any potential effect on the development of infertility. In one of these studies, performed by Ozturk *et al.* (2016), the expression levels of *PABPC1*, *EPAB*, and *PABPC3* mRNAs and proteins were examined in patients with non-obstructive azoospermia, including hypospermatogenesis, round spermatid arrest, spermatocyte arrest, and Sertoli cell-only syndrome [62]. Hypospermatogenesis is defined as a marked reduction of sperm production from some seminiferous tubules. On the other hand, spermatogenic activity is arrested largely at round spermatids or spermatocytes in round spermatid arrest and spermatocyte arrest cases, respectively. Only Sertoli cells are present in the seminiferous tubules of patients diagnosed as Sertoli cell-only syndrome. In this study, it was found that the mRNA and protein

Table 1. The general features of Epab, Pabpc1, and Pabpc2

Pabp protein	Structure	Binding site on RNA sequence	Knockout phenotype	Subcellular localization	Fundamental functions	Interacting mRNAs	Interacting proteins/ complexes
Epab	• PRM1, 2, 3, 4 motifs • Proline-rich region • PABC domain	• Poly(A) tail • AU-rich sequences	Male: Fertile Female: Infertile	Cytoplasm	• Cytoplasmic polyadenylation • Stimulates translation • Inhibits deadenylation	*RINGO/Spy, Areg, Ereg, Btc, Ptgs2, Has2, Tnfaip6, Msy2, Ccnb1, c-Mos, Dazl*	Cpab, Ringo, Eif4g, Pum2, Dazl, Ccnb1, Pabpc1, Paip1/Polyribosomes
Pabpc1	• PRM1, 2, 3, 4 motifs • Proline-rich region • PABC domain	• Poly(A) tail • 3' UTR region • Poly(U) sequences	Yeast: Lethal	Cytoplasm Nucleus	• Stimulates translation • mRNA turnover • Translational repression • miRNA-based regulation	*Actb, Gapdh, Sycp3, Acrbp, Smcp, Prm1 and 2*	Eif4g, Pabpc1, Pabpc2, Paip1, Paip2, Piwil1, Epab/ Polyribosomes, Risc
Pabpc2	• PRM1, 2, 3, 4 motifs • Proline-rich region • PABC domain	• Poly(A) tail	Male: Fertile	Cytoplasm Nucleus	• Enhances translation • Translational repression • mRNA metabolism • mRNA degradation	*Actb, Gapdh, Sycp3, Acrbp, Smcp, Prm1 and 2*	Paip1, Paip2, Pabpc1, Pabpc2, Piwil1/ Polyribosomes, Chromatoid bodies

This table introduces the typical features of PABPs: their structure, RNA-binding activities, subcellular localization, and their known mRNA and protein interactions. There are similarities between PABPs, such as their common roles in translation control of the mRNAs via binding to their poly(A) tails, as well as differences, which most likely originate from their intracellular functional differences. NA, Not analyzed; *Acrbp*, proacrosin-binding protein; *Smcp*, sperm mitochondria-associated cysteine-rich protein; *Prm1 and 2*, protamines 1 and 2; *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase; *Actb*, beta-actin; *Pum2*, pumilio 2; *Dazl*, deleted for azoospermia-like; Piwil1, piwi-like protein 1; *Areg*, amphiregulin; *Ereg*, epiregulin; *Btc*, betacellulin; *Ptgs2*, prostaglandin synthase 2; *Has2*, hyaluronan synthase 2; *Tnfaip6*, tumor necrosis factor α-induced protein 6; Ccnb1, cyclin B1; Risc, RNA-induced silencing complex; Ringo/Spy, Rapid inducer of G2-M in oocytes/Speedy; Msy2, Y-box binding protein 2; c-Mos, Moloney sarcoma oncogene.

levels of PABPs are significantly decreased from hypospermatogenesis to Sertoli cell-only groups. These findings suggested that *PABPC1*, *EPAB*, and *PABPC3* may play roles in the translational control of mRNAs stored during spermatogenesis and that potential alterations in their expressional profiles may play roles in the development of male infertility in non-obstructive azoospermia patients [62]. In isolated spermatogenic cells, it was revealed that spermatocytes and round spermatids obtained from the hypospermatogenesis group had significantly higher expression levels of *PABPC1*, *EPAB* and *PABPC3* mRNA and proteins than the spermatocyte and round spermatid fractions isolated from the RS arrest or SC arrest groups [62]. Since there have not been many studies on the possible relationship between the development of male infertility and *PABP* gene expression, it is difficult to interpret the potential roles of PABPs in male infertility.

New studies are required to determine the molecular biological roles of PABPs during spermatogenesis, as well as in fertilization and early embryo development. Furthermore, the potential relationship between the development of male infertility and genetic changes of *PABP* genes such as mutations and polymorphisms should be examined in oocytes and early embryos. Results of previous studies suggest that PABPs may contribute to three processes: First, sperm cells carry thousands of distinct RNAs, including mRNAs [74, 75], to the oocyte that is being fertilized. Since the primary functions of PABPs are protecting mRNAs from undergoing premature degradation and regulating their translation activities, it is possible that PABPs may play a role in protecting the RNAs and/or mRNAs in sperm cells while being transported to oocytes that are being fertilized. It is still unknown what the possible functions of PABPs in the protection and translational regulation of these RNAs during fertilization and early embryonic development would be. Second, we do not know whether any PABPs coming from sperm cells enter mature oocytes during fertilization or what their fate in the zygote and onward would be if they do. Third, the strict translational regulation in spermatogenic cells during spermatogenesis seems to play a crucial

role in generating competent spermatozoa capable of succeeding in fertilizing mature oocytes, as well as in early embryogenesis and the outcome of pregnancy. PABPs during the process of producing competent sperm cells may have implications other than merely regulating translational activity.

Conclusion

Evolutionarily conversed PABPs play crucial roles in mRNA biogenesis and stabilization, as well as the translational regulation of mRNAs previously stored during spermatogenesis, and they exhibit fundamental functional differences (Table 1). Some PABPs seem to be functionally redundant and dispensable for transitional control in spermatogenic cells during spermatogenesis. Although *Epab* or *Pabpc2* knockout mice models have revealed that the lack of these PABPs does not negatively affect spermatogenic activity, the potential effects of the other PABP isoforms on the spermatogenesis process are not well known; this ought to be explored in detail in future studies. The findings obtained from new studies would elucidate the molecular background of male infertility, which may stem from altered PABP expression. Finally, the potential interactions of PABPs with mRNAs that undergo translational regulation and with other cytoplasmic proteins that play roles in various types of cellular events should be clearly characterized in order to understand all existing functions of PABPs in spermatogenic cells during spermatogenesis.

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