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REVIEW ARTICLE

The translational functions of embryonic poly(A)-binding protein during gametogenesis and early embryo development

Saffet Ozturk

Department of Histology and Embryology, Akdeniz University School of Medicine, Antalya, Turkey

Correspondence
Saffet Ozturk, Department of Histology and Embryology, Akdeniz University School of Medicine, Campus, 07070, Antalya, Turkey.
Email: sozturk@akdeniz.edu.tr

Abstract

Embryonic poly(A)-binding protein (EPAB) is an RNA-binding protein that binds to the poly(A) tails and AU-rich element at the 3′ ends of messenger RNA (mRNAs). The main functions of EPAB are to protect stored mRNAs from undergoing deadenylation and subsequent degradation and to be involved in their translational regulation during spermatogenesis, oogenesis, and early embryogenesis. Following the first characterization of Epab in the *Xenopus* oocytes and early embryos, spatial and temporal expression and potential roles of the *Epab* gene have been determined in the vertebrate germ cells and early embryos. In this review, we have comprehensively evaluated all studies in this field and discussed the particular functions of EPAB in the spermatogenic cells, oocytes, early embryos, and somatic cells in vertebrates.

KEYWORDS
early embryo, EPAB, oocyte, spermatogenic cell, translational regulation

1 | INTRODUCTION

Spermatogenesis takes place in the seminiferous tubules of testes and is basically divided into three main stages: spermatocytogenesis, meiosis, and spermiogenesis (Chocu, Calvel, Rolland, & Pineau, 2012). In the spermatocytogenesis stage, spermatogonial stem cells (SSCs) experience a series of mitotic divisions, and some of them differentiate into type B SSCs having cytoplasmic bridges (de Rooij, 2001). In the meiotic stage, type B SSCs enter the first round of meiotic division after which they are defined as primary spermatocytes. The primary spermatocytes complete the first meiotic division to produce secondary spermatocytes that undergo a second round of meiotic division to form haploid round spermatids. The round spermatids experience remarkable morphological and physiological changes such as nuclear condensation, flagella and acrosome formation, and mitochondrial reorganization during spermiogenesis stage. Eventually, specialized four male germ cells, spermatozoa or sperm cells, are generated at the end of each spermatogenic activity (Larson, Kopania, & Good, 2018).

Oogenesis is briefly defined as the transformation of oogonial stem cells (OSCs) into mature oocytes. Once OSCs enter the meiotic division, they are known as oocytes. Meanwhile, squamous pre-granulosa cells surround the primary oocytes, which are arrested at the diplotene of the first meiotic prophase division. Thus, primordial follicles localized at the cortical region of the ovary appear at approximately the 16th-week of gestation in humans (Oktem & Urman, 2010). Before puberty, a subset of primordial follicles is induced to grow in a gonadotropin-independent manner in humans (Peters, Byskov, & Grinsted, 1978). The pre-granulosa cells enclosing the primary oocytes in the primordial follicles transform into cuboidal cells, and thereby generating primary follicles. At the end of consecutive mitotic divisions of granulosa cells, late primary follicles involving more than 2 layers of granulosa cells are formed. When small spaces among granulosa cells begin to occur, these follicles are then defined as secondary follicles. The later stages of follicular development proceed in a gonadotropin-dependent manner because the receptors of either follicle stimulating hormone (FSH) in the secondary follicles or luteinizing hormone (LH) in the antral follicles emerge in the granulosa and theca cells (Kishi, Kitahara, Imai, Nakao, & Suwa, 2018). The small spaces among the granulosa cells constitute a big antrum, and primary oocytes surrounded by cumulus cells reside in an eccentric position. Eventually, Graafian follicles (also known as preovulatory or antral follicles) emerge. Following an LH surge in humans or an increase of progesterone in *Xenopus*, meiotic

activity is resumed and secondary oocytes arrested at the metaphase II phase of the second meiotic division are ovulated with surrounding cumulus cells (Erickson & Shimasaki, 2001). Fertilization of mature secondary oocytes with viable spermatozoa results in the production of 1-cell embryos (also known as zygotes) with male and female pronuclei. The 1-cell embryos undergo sequential mitotic divisions known as "cleavage" to produce 2-cell, 4-cell, 8-cell, 16-cell, morula, and blastocyst stage embryos, respectively (Assou et al., 2011).

2 | FROM TRANSCRIPTION TO TRANSLATION IN THE GERM CELLS AND EARLY EMBRYOS

The developmental processes, which include meiotic progression, oocyte to embryo transition, later spermatogenesis and early embryogenesis, require timely and strictly regulated transcriptional and translational control (Braun, 2000; Susor & Kubelka, 2017). For this purpose, newly transcribed precursor (pre)-messenger RNAs (mRNAs) in the nucleus undergo a number of modifications such as capping, polyadenylation, and splicing in the germ cells and early embryos as is observed in eukaryotic cells (Barckmann & Simonelig, 2013; Piccioni, Zappavigna, & Verrotti, 2005). While transcription continues, the 5′ ends of mRNAs are capped either with a 7-methylguanosine residue (m7G) or by adding more methyl groups to the adjacent guanosines, a process known as simply "capping" (Warminski, Sikorski, Kowalska, & Jemielity, 2017). The basic function of capping is to protect the mRNAs from 5′ to 3′ exonucleases attack. At the end of transcription, the cleavage and polyadenylation specificity factor (CPSF) binds to the cleavage and polyadenylation signal (AAUAAA or AUUAAA) at the 3′ ends to stimulate cleavage at the 15-30 nucleotides downstream of this sequence (Neve, Patel, Wang, Louey, & Furger, 2017). Then, a poly(A) tail is generated by the process of adenylation, in which adenosine monophosphates (AMPs) are attached each to other with a phosphodiesterase bond. In this process, the phosphodiester bond between a hydroxyl side chain of the first AMP and phosphate group of the second AMP is formed. This process is called as adenylation. The addition of multiple AMPs to the newly transcribed mRNAs is defined as polyadenylation (Edmonds, 2002). The addition of a poly (A) tail to the cleaved 3′ ends of the newly transcribed mRNAs as a result of polyadenylation is catalyzed by the enzyme poly(A) polymerase (PAP) in the nucleus. The polyadenylation also contributes to nuclear export, translation and maintaining the integrity of the modified mRNAs (Stewart, 2019). Importantly, mRNAs include untranslated regions (UTR) at both 5′ and 3′ ends, and the UTR regions play roles in the modifications and translational regulation of the mRNAs (Barrett, Fletcher, & Wilton, 2012). The last modification on the pre-mRNAs is to remove the intervening sequences under the action of spliceosome complex (Will & Luhrmann, 2011). As a consequence, the modified mature mRNAs are exported from the nucleus to the cytoplasm thorough the nuclear pore complex mediated by nuclear export factors (NXFs; Erkmann & Kutay, 2004).

In the cytoplasm, translation of the exported mature mRNAs takes place in two ways: global and specific (reviewed in Nandagopal and Roux (2015)). The continuous protein synthesis for the basic cellular metabolisms is being carried out by the global translation (Sonenberg & Hinnebusch, 2009). Conversely, specific translation of the stored mRNAs (discussed below in detail) occurs at certain points under the control of intracellular cascades in germ cells and early embryos (Friday & Keiper, 2015; Jansova, Tetkova, Koncicka, Kubelka, & Susor, 2018). Translational complex composed of the eukaryotic translation initiation factor (eIF)4A, eIF4B, eIF4E, and eIF4G, and the remaining translation-associated factors such as eIF1, eIF1A, eIF2, eIF3, and eIF5 are formed in both ways to induce the translational activity of mature mRNAs (Burgess & Gray, 2010). Following translation of the target mRNAs in the sufficient levels, they normally undergo a degradation process; however, only a subset of mRNAs dissociated from polysomes undergoes degradation in the mouse oocytes (Chen et al., 2011). The mRNA degradation is regulated with *cis*-acting sequences and *trans*-acting factors in response to developmental, environmental, and metabolic signals (reviewed in Tourriere, Chebli, and Tazi (2002)). The first step of the 3′ to 5′ mRNA degradation is deadenylation, in which the poly(A) tails are shortened by the enzyme poly(A)-specific ribonuclease (PARN). Subsequently, the deadenylated mRNAs are degraded from 3′ ends by the exosomes. In the 5′ to 3′ mRNA decay pathway, decapping enzymes such as decapping 1 (DCP1) and 2 (DCP2) remove the cap structure. This results in the degradation of the mRNAs through 5′-3′ exoribonuclease 1 (XRN1) (Tourriere et al., 2002). It is important to note that in the specific translation, the mature mRNAs are deposited in the silencing complexes such as ribonucleoprotein particles (RNPs) after partial deadenylation. In addition, the stored mRNAs undergo timely controlled translational activation in germ cells and early embryos following the period of transcriptional repression (Stebbins-Boaz & Richter, 1997).

During spermatogenesis in vertebrates, transcriptional activity ceases largely due to condensed chromatin structure caused by special protamines at the mid-spermiogenesis (Kierszenbaum & Tres, 1978; Steger, 1999). Similarly, transcription is restricted once meiotic activation of primary oocytes underway during later periods of oogenesis (Bachvarova, 1985; De La Fuente et al., 2004) until embryonic genome activation (EGA) takes place at the 2-cell in mice (Flach, Johnson, Braude, Taylor, & Bolton, 1982) and the 4–8 cell stage embryos in humans (Braude, Bolton, & Moore, 1988). For this reason, the mRNAs required after transcriptional silencing in the later stages of spermatogenesis and oogenesis even during early embryogenesis, are transcribed in the early stages and stored as RNPs (Elliott, 2003). Translational activation of the stored mRNAs is mainly carried out in either cap-dependent or cap-independent ways. In the cap-dependent translation, various types of eukaryotic translation initiation factors (e.g. eIF4E and eIF4G), poly(A)-binding proteins (PABPs), and the remaining translation-related factors contribute to forming a "closed loop" mRNA structure by establishing a bridge between the poly(A) tail at the 3′ end and m7G at the 5′ end (Vicens, Kieft, & Rissland, 2018). The closed loop formation facilitates

translation of the target mRNAs and protects them from any nuclease attack (Burgess & Gray, 2010). In the cap-independent translation mechanism, special structures such as hairpins, pseudo-knots, internal ribosome entry sites, and upstream open reading frames at the 3′ or 5′ UTRs of the target mRNAs largely regulate the translational activity (Keiper, 2019; Wang, Parisien, Scheets, & Miller, 2011).

As a subset of mRNAs experience cap-dependent translation during spermatogenesis, oogenesis and early embryogenesis, PABPs especially embryonic poly(A)-binding protein (EPAB) play key roles in the translational regulation of the stored mRNAs upon binding to their poly(A) tails and ARE (AU-rich element; AUUUA). As is well known, the exported mRNAs with long poly(A) tails from nucleus to cytoplasm undergo poly(A) shortening from 250 adenosines (As; Eckmann, Rammelt, & Wahle, 2011) to 30 As (Korner & Wahle, 1997), and they are stored as translationally suppressed RNPs. To preclude the unfavorable translational activation of the cytoplasmic polyadenylation element (CPE)-containing mRNAs in *Xenopus*, Maskin establishes a bridge between cytoplasmic polyadenylation element binding protein 1 (Cpeb1) and Eif4e to prevent formation of translation initiation complex (reviewed in Reyes and Ross (2016)). Upon Cpeb1 phosphorylation on Ser174 by Aurora A kinase due to progesterone stimulation in *Xenopus*, Maskin leaves from the complex, resulting in translation induction (Mendez et al., 2000). Notably, it has been reported that Aurora A kinase mediate CPEB1 activating phosphorylation in the mouse oocytes (Hodgman, 2001), but not in the porcine oocytes (Komrskova et al., 2014). The phosphorylation of Cpeb1 leads to increasing its affinity to Cpsf and recruits germ line development 2 (Gld2; Richter, 2007) so that the shortened poly(A) tails of the maternally stored mRNAs are elongated by cytoplasmic polyadenylation as a first step of translational activation in *Xenopus* oocytes (Radford, Meijer, & de Moor, 2008). In addition to Cpeb1, other factors such as symplekin (Sympk), Cpsf, and Gld2, an atypical cytoplasmic poly(A) polymerase function in the translational regulation (Barnard, Ryan, Manley, & Richter, 2004). It should be noted that, there is also another translational activation mechanism possibly independent of cytoplasmic polyadenylation in the *Xenopus* oocytes in which deleted in azoospermia-like (Dazl) is involved; this is explained in the later section (Collier, Gorgoni, Loveridge, Cooke, & Gray, 2005). This mechanism is also defined in zebrafish germ cells, which is referred to as poly(A)-tail-independent noncanonical translation (PAINT; Mishima, 2018). The PAINT is briefly defined as the Eif4f- and Pabp-free ribosome loading pathway (Mishima, 2018).

3 | STRUCTURE OF EMBRYONIC POLY(A)-BINDING PROTEIN

In vertebrates, EPAB (also known as ePabp, ePab and Pabpc1-like) is an RNA-binding protein that binds to ARE and poly(A) tails at the 3′ ends of mRNAs (Voeltz, Ongkasuwan, Standart, & Steitz, 2001). Basically, EPAB is composed of four RNA recognition motifs (RRMs) at the N-terminus, proline-rich region, and PABC domain at the C-terminus (Mangus, Evans, & Jacobson, 2003; Figure 1). The highly conserved RRM1, 2, 3, and 4 motifs play a role in inducing translational activity of the mRNAs by associating with approximately every 25 adenosines (Sachs, Davis, & Kornberg, 1987; Voeltz et al., 2001). In addition to their mRNA binding ability, the RRMs can also interact with Eif4g (Gray, Coller, Dickson, & Wickens, 2000) and Pabp-interacting protein 1 (Paip1; Roy et al., 2002) proteins (Mangus et al., 2003; Figure 1). RRM1 and RRM2 link with the 29-amino acid of N terminal region of eIF4G (Imataka, Gradi, & Sonenberg, 1998) to initiate translation of the mRNAs having a CPE sequence (Kim & Richter, 2007). Notably, a recent study revealed that the number and localization of the CPE sequence determined by the polyadenylation

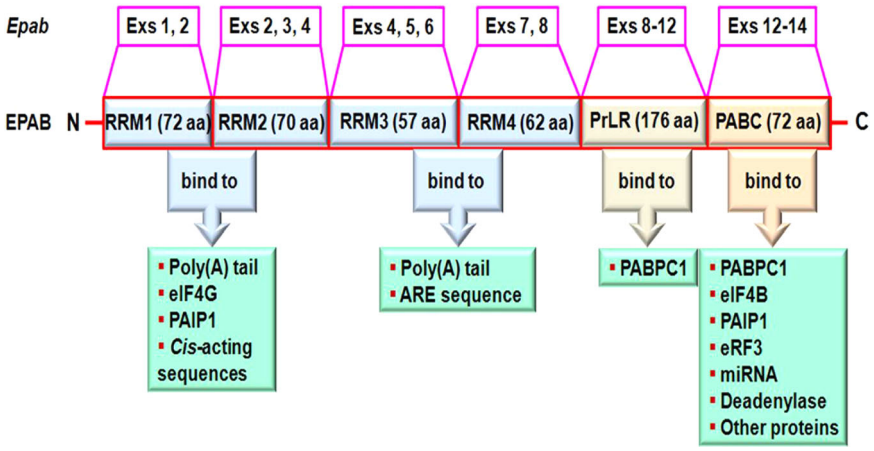


FIGURE 1 The structure of embryonic poly(A)-binding protein (EPAB) in *Xenopus*, mice and humans. The *Epab* mRNA is composed of 14 exons, and each exon contributes to encoding the EPAB domains. Basically, EPAB consists of four RNA recognition motifs (RRMs), proline-rich linker region (PrLR) and poly(A)-binding protein C-terminal domain (PABC) domains. Each domain enables the binding of different sequences and/or factors to accomplish their specific missions. aa, amino acid; ARE, AU-rich element; eRF3, eukaryotic release factor 3; Exs, exons; eIF4B and 4G, eukaryotic initiation factor 4B and 4G; PABPC1, poly(A)-binding protein cytoplasmic 1; PAIP1, PABP-interacting protein 1; miRNA, microRNA [Color figure can be viewed at wileyonlinelibrary.com]

signal seem to play critical roles in controlling the translation of maternal mRNAs in mouse oocytes (Dai et al., 2019). As is well-known in the *Xenopus* oocytes, Cpeb1 binds to the CPE sequence at the 3 UTR to mediate poly(A) tail stabilization and translation regulation (Kim & Richter, 2007). In addition to RRM1 and 2, the C-terminal of the Pabp protein maintains interactions with Paip1 to stimulate translational activity by promoting mRNA circularization (Lv, Zhang, & Gao, 2014; Wilkie, Gautier, Lawson, & Gray, 2005). The RRM3 and RRM4 are capable of binding to the ARE sequence of mRNAs to control the translational activity (Gray et al., 2000). On the other hand, the PABC domain partially participates in activating translational activity (Mangus et al., 2003; Wilkie et al., 2005) and in protein–protein interactions with, for example, the HECT family of ubiquitin ligases, Paip1, PABP-interacting protein 2 (Paip2), and eukaryotic release factor 3 (Erf3) proteins (Kozlov et al., 2001). The proline-rich region located between RRM4 and PABC domain are required for Pabp–Pabp interaction to repress translational activity of the reporter mRNAs (Melo et al., 2003; Figure 1).

It is noteworthy that Epab includes similar structural domains with ubiquitously expressed somatic poly(A)-binding protein cytoplasmic 1 (Pabpc1), therefore they can interact with several similar factors in vertebrates (Wilkie et al., 2005). However, RRM3 and proline-rich linker region of both Epab and Pabpc1 proteins display structural divergences (Voeltz et al., 2001). The potential effect(s) of this divergence on their functional features remain elusive. It has been revealed that Epab and Pabpc1 proteins are able to bind to the same reporter mRNA to activate its translation (Wilkie et al., 2005). Another PABP protein, poly(A)-binding protein cytoplasmic 5 (Pabpc5), has four non-identical RRM motifs, but there are no other domains defined in Epab and Pabpc1 (Wilkie et al., 2005). The *PABPC5* gene is found to be expressed in the human somatic tissues as well as in the ovary (Blanco et al., 2001). The nuclear Pabp protein involving poly(A)-binding protein nuclear 1 (Pabpn1) and embryonic poly(A)-binding protein 2 (Epabp2) possess only one RRM motif, and there are no the remaining domains (Wilkie et al., 2005). The PABPN1, also known as nuclear PABP, specifically binds to the poly(A) tails of pre-mRNAs when they are exported into cytoplasm following maturation (Wahle, 1991). The Epabp2 protein, cytoplasmic form of Pabpn1, is also capable of interacting with the poly(A) tails of mRNAs in the *Xenopus* oocytes and early embryos (Good, Abler, Herring, & Sheets, 2004) and in the mouse oocytes and early embryos as well (Good et al., 2004). Taken together, along with some similarities in the domains, PABP proteins exhibit structural differences possibly based on the functional divergence.

Epab associates with Pabpc1, suggesting that they enable to bind the same mRNAs at the same time to modulate their translation. Also, the interaction between these two Pabp proteins contributes to providing strong binding to the poly(A) tails of mRNAs (Wilkie et al., 2005). They can consistently compensate for each other's functions in *Xenopus* (Cosson et al., 2002) and possibly in mice (Uysal & Ozturk, 2019). The possible interactions and compensations of each other's functions of mammal PABP proteins should be analyzed in more detail to understand their capacity to regulate the translation of stored mRNAs.

The phylogenetic analysis revealed that *EPAB* is expressed in birds, fish, frogs, and mammals (Wilkie et al., 2005). The mouse *Epab* gene localized on chromosome 2, includes 14 exons, and encodes a 608 amino acids (aa) protein (Seli et al., 2005; Figure 1). Also, *Epab* mRNA undergoes an alternative splicing process to create the transcript without exon 11 that encodes a 561 aa isoform in the mouse oocytes (germinal vesicle [GV] and metaphase II [MII] oocytes) and early embryos (1-cell and 2-cell embryos), revealed by using exons 10.5 forward (F)-12 reverse (R) primers (Seli et al., 2005). The production of *Epab* transcripts is mainly regulated by the exonic splicing enhancers generated by the single nucleotide polymorphisms (SNPs) in the related exons (Seli, Yaba, Guzeloglu-Kayisli, & Lalioti, 2008). In the mouse ovary, three alternative splicing of mRNAs lacking exon 10, exon 13, and both exons are present in addition to the full-length transcript, documented by using exons 8F-14R primers (Seli et al., 2008). The alternatively spliced form of *Epab* mRNA lacking exon 10 (encoding PABC domain) is similarly produced by an exonic splicing enhancer and exhibits monoallelic expression in the mouse oocytes. It is important to note that there are no differences in the relative expression levels among the alternative spliced *Epab* mRNAs (Seli et al., 2005).

The human *EPAB* gene which is located on chromosome 20, has 14 exons and encodes a 619-aa protein (Guzeloglu-Kayisli et al., 2008; Sakugawa et al., 2008). Similarly, the *EPAB* gene undergoes alternative splicing by lacking exon 9 or exons 9 and 10 in the human ovary (Guzeloglu-Kayisli et al., 2008). There is a cryptic donor acceptor site in the middle of exon 8, and this leads to the production of a truncated protein not including in the PABC domain (Guzeloglu-Kayisli et al., 2014). It is important to note that human EPAB protein shows 77% identity (72% was found in another study [Sakugawa et al., 2008]) and 84% similarity to mouse EPAB, and 72% identity and 83% similarity to *Xenopus* Epab (Guzeloglu-Kayisli et al., 2008). By contrast, researchers have found 65% identity and 76% similarity in the amino acid residues of the *Xenopus laevis* and mouse EPAB proteins (Wilkie et al., 2005). The high divergences between *Xenopus*, mouse, and human EPAB proteins most likely originate from having distinctly different amino acid sequences in their C-terminal regions (Guzeloglu-Kayisli et al., 2008). Also, the possible functional differences of the EPAB protein that emerged during evolutionary development from *Xenopus* to human may be another reason for this divergence.

4 | THE TRANSLATIONAL FUNCTIONS OF EPAB IN THE OOCYTES AND EARLY EMBRYOS

Basically, the Epab protein binds to either ARE (Voeltz et al., 2001) or poly(A) tails at the 3′ ends of the stored mRNAs to stimulate their translation (Wilkie et al., 2005). Also, Epab protects poly(A) tails from deadenylase such as Parn or other nucleases (Kim & Richter, 2007). In normal conditions, the Parn enzyme is more active than the cytoplasmic poly(A) polymerase Gld2; therefore, the processed

mature mRNAs exported into cytoplasm undergo deadenylation to repress their translation (Kim & Richter, 2006). In other words, there is a balance between the Parn and Gld2 activities that serve to regulate the cytoplasmic deadenylation/polyadenylation of the processed and stored mRNAs. Cpeb1 phosphorylation during oocyte maturation leads to the expulsion of the Parn enzyme from the ribonucleoprotein complex. This results in poly(A) elongation by Gld2 (Kim & Richter, 2006; Figure 2a). Following Parn expulsion, Epab binds to the poly(A) tails of the stored mRNAs undergoing polyadenylation and then translation. In addition, Epab interacts with the polysomes to regulate the translation of the target mRNAs (Wilkie et al., 2005). At the same time, the interaction of Epab with Eif4f complex by the way of associating with Eif4g contributes to disrupting the repression complex established by Maskin-Eif4e (Cao & Richter, 2002). This allows Eif4g to link with Eif4e and thereby initiate translational activity (Cao & Richter, 2002; Figure 2a). The Maskin protein notably includes a peptide sequence conserved among the Eif4e binding proteins. It is expressed in the *Xenopus* oocytes and embryos, but not in several somatic tissues such as those in the heart, brain, muscle, and liver (Stebbins-Boaz, Cao, de Moor, Mendez, & Richter, 1999). Thus, in addition to Cpeb1, Maskin interacts with Eif4e to repress the translation of CPE-containing mRNAs by preventing the association between Eif4e and Eif4g (Stebbins-Boaz et al., 1999). During oocyte maturation, Maskin dissociates from Eif4e to facilitate translation of CPE-containing mRNAs (Stebbins-Boaz et al., 1999; Figure 2a). It is important to note that there is no human ortholog of Maskin defined as yet, and which molecule or molecules carry out this mission in human oocytes remains unclear. Overall, these findings suggest that Epab contributes to cytoplasmic polyadenylation-dependent translation in cooperation with other translation-related factors (Cosson et al., 2002). Cao and Richter (2002) reported that Epab is consistently involved in progesterone-induced expression of *cyclin b* mRNA having long poly(A).

The Epab protein also functions in activating the rapid inducement of G2-M (*ringo)*/speedy (*spy*) mRNA by means of a polyadenylation-independent mechanism, in which Pumilio 2 (Pum2)/Dazl/Epab complex is formed in *Xenopus* oocytes (Padmanabhan & Richter, 2006; Figure 2b). In immature *Xenopus* oocytes, Pum2 associates with Pumilio-binding element (PBE) at the 3′ UTR of *ringo/spy* mRNA to repress its premature translation. Upon oocyte maturation induced by progesterone in *Xenopus*, Pum2 reversibly displaces from the translational complex (Pum2-Dazl-Epab) of the *ringo/spy* mRNA to provide its translation (Padmanabhan & Richter, 2006; Figure 2b). This complex also plays a key role in the stabilization of the stored mRNAs in immature *Xenopus* oocytes (Padmanabhan & Richter, 2006). Ringo/Spy is of course required for activating cyclin-dependent kinases (Cdks), being involved in the Cpeb1-dependent cytoplasmic polyadenylation (Padmanabhan & Richter, 2006). In the cytoplasmic polyadenylation, Epab also participates in the complex composed of the Cpeb1/Sympk/Cpsf to provide translation of the stored mRNAs in *Xenopus* oocytes (Kim & Richter, 2007).

In the *Xenopus* oocytes, Epab is known to bind the ARE and poly (A) tails to protect the mRNAs independent of having ARE or are not

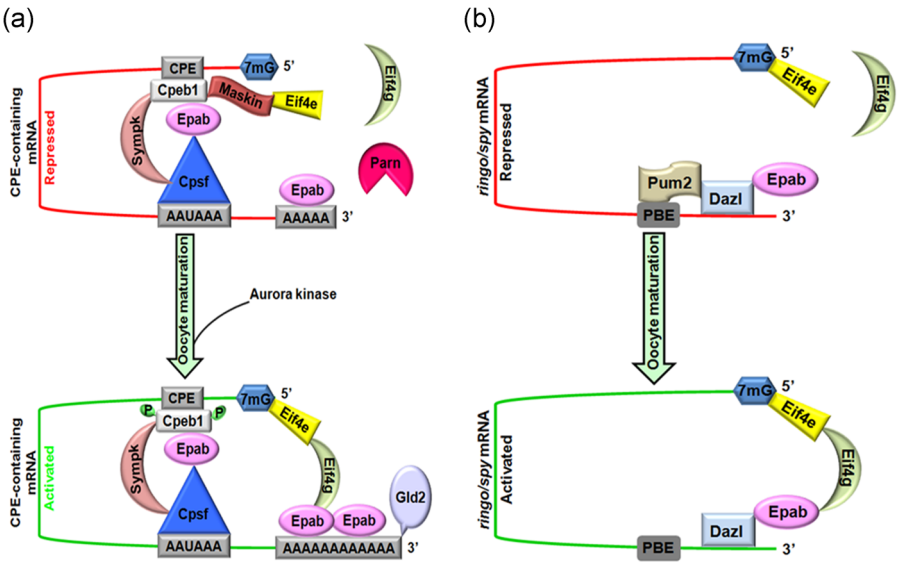


FIGURE 2 Translation control of cytoplasmic polyadenylation element (CPE)-containing mRNAs and *ringo/spy* mRNA in *Xenopus*. (a) Translation of the repressed CPE-containing mRNAs having short poly(A) tail takes place following phosphorylation of Cpeb1 by Aurora kinase. The Cpeb1 phosphorylation results in removing Maskin from the complex so that translation of the target mRNAs with long poly(A) tails bound by Epab appears during oocyte maturation and early embryogenesis. (b) Upon oocyte maturation, the *ringo/spy* mRNA translation is carried out after removing Pum2 from the repression complex. Thus, the translation complex including Dazl-Epab-Eif4g-Eif4e provides the *ringo/spy* mRNA translation independent of polyadenylation. AAAAAA, poly(A) tail; A, adenosine; AAUAAA, cleavage and polyadenylation signal; Cpeb1, cytoplasmic polyadenylation element binding protein 1; Cpsf, cleavage and polyadenylation specificity factor; Eif4e and 4g, eukaryotic initiation factor 4e and 4g; Epab, embryonic poly(A)-binding protein; Gld2, germ line development 2; p, phosphorylation; Parn, poly(A)-specific ribonuclease; Sympk, symplekin; U, uracil [Color figure can be viewed at wileyonlinelibrary.com]

in vitro (Voeltz et al., 2001). The number of AREs, not bound by Epab positively correlates with the rate of deadenylation (Voeltz et al., 2001). The immunodepletion of Epab consistently results in ARE-mediated and default deadenylation in the *Xenopus* oocyte extracts (Voeltz et al., 2001). The addition of Epab to the depleted oocyte extracts results in inhibiting ARE-mediated deadenylation. In *Xenopus*, Epab expression is at the similar levels during early development from stage IV oocytes to 30-hr embryo, and there is no expression detected in the somatic tissue culture (Cosson et al., 2002; Voeltz et al., 2001); however, kidney, heart, and testis tissues express its mRNA (Wilkie et al., 2005). Although Epab expression is at the detectable levels in the stage I oocytes, it is significant that it reaches high levels in the stage VI oocytes (Wilkie et al., 2005). Epab resides in the animal pole of the *Xenopus* immature oocytes at a higher level when compared with the vegetal pole (Friend et al., 2012). In the stage IV oocytes, it localizes in the cytoplasmic region and there is no expression in the nuclear region (Voeltz et al., 2001). It is important to recall that the subcellular localization of Epab takes place independent of its phosphorylation status (Friend et al., 2012). As a result, *Xenopus* oocytes and early embryos express Epab, and it functions in the poly(A) tail stabilization and translational regulation of the stored mRNAs (reviewed in Esencan, Kallen, Zhang, and Seli, (2019)). The distinct subcellular localization of Epab in the *Xenopus* oocytes at different stages may derive from high levels of storing maternal mRNAs during oocyte development.

For the purpose of translational control of the stored mRNAs, Epab interacts with Dazl in the *Xenopus* oocytes independent of progesterone treatment (Collier et al., 2005; Padmanabhan & Richter, 2006; Voeltz et al., 2001) so that Pabps recruitment to the mRNAs undergoing translation is provided (Collier et al., 2005). The binding of the recruited Epab to the elongating poly(A) tails induces dissociation of Maskin from the translation complex, resulting in translational activation in the *Xenopus* egg extracts (Cao & Richter, 2002). Upon induction of *Xenopus* oocyte maturation by progesterone, Aurora A/Eg2 kinase phosphorylates the Pum2 protein, leading to *ringo/spy* mRNA translation (Padmanabhan & Richter, 2006). Overall, the Dazl–Pum2-Epab complex functions in suppressing the translational activity of *ringo/spy* mRNA (Padmanabhan & Richter, 2006). The phosphorylation of Pum2 leads to the disruption of the Dazl-Pum2-Epab complex, which results in the translation of the *ringo/spy* mRNA. This translation, in turn, causes meiotic resumption of the prophase I-arrested oocytes (Padmanabhan & Richter, 2006; Figure 2b).

In the immature *Xenopus* oocytes, CPE-containing mRNAs such as cyclin b1 (*ccnb1*) are maintained in a dormant state during which Cpeb1 binds to either the CPE element or Maskin. In this complex, Maskin interacts with the Eif4g to prevent the association between Eif4g and Eif4e factors for the purpose of inhibiting the translation initiation complex (Cao & Richter, 2002). After increasing progesterone level in *Xenopus*, CPE-containing mRNAs are translated after dissociation of Maskin from Eif4e to facilitate the interaction between Eif4e and Eif4g factors during oocyte maturation (Cao & Richter, 2002). The translational activity requires not only Cpeb1 phosphorylation but also binding of Epab to the elongating poly(A) tails. It is important to note that the interaction between Epab and Eif4g contributes to dissociating Eif4e from Maskin, which leads to the translation of the CPE-containing dormant mRNAs such as *cyclin b1* (Cao & Richter, 2002). On the other hand, Epab associates with the Cpeb1-Sympk-Cpsf (Kim & Richter, 2007) and Dazl-Pum2 (Padmanabhan & Richter, 2006) complexes to stabilize the mRNAs by preventing poly(A) tails from shortening (Voeltz et al., 2001) and translational regulation of these mRNAs in the *Xenopus* oocytes (Wilkie et al., 2005; Figure 2a,b). The potential interactions of Epab with other translation-related proteins and intracellular proteins still need to be evaluated in mammalian oocytes and early embryos.

Consistent with known functions of Epab, the morpholino-mediated knockdown of the *epab* gene expression leads to phenotypic defects in the anterior-posterior axis formation, movement defects and embryonic lethality by stage 35 in *Xenopus laevis* (Gorgoni et al., 2011). Also, the cross-rescue treatment with the *epab* mRNA cannot prevent the omission of the *pabpc1* gene from the phenotypes derived in this way. This suggests that Pabps seem to exhibit different roles in *Xenopus* (Gorgoni et al., 2011).

Friend et al. (2012) reported that Epab undergoes dynamic phosphorylation in the four residues (Ser^{460}, Ser^{461}, Ser^{464} and Thr^{465}) necessary for oocyte maturation in *Xenopus laevis*. This phosphorylation is required for cytoplasmic polyadenylation, but not for translational activation of the stored mRNAs and subcellular localization of Epab in the immature stage IV *Xenopus* oocytes. The rate of phosphorylated Epab is consistently increased during oocyte maturation. The hyperphosphorylated Epab is capable of binding to the complexes related to mRNA translation and polyadenylation (Friend et al., 2012). Conversely, the hypophosphorylated Epab cannot associate with the polysomes or cap complexes (Friend et al., 2012). These findings suggest that Epab undergoes posttranslational modifications such as phosphorylation during oocyte maturation, most likely for the purpose of regulating the translational activity of the stored mRNAs. However, it remains unknown whether the same or greater modifications occur during oogenesis or even during spermatogenesis in mammalians.

In mice, *Epab* is transcribed in the ovary and testis, but not in the somatic tissues (Seli et al., 2005; Wilkie et al., 2005). In the ovary, it resides in the cytoplasm of oocytes located in the primary and preantral follicles; there is no *Epab* expression in the granulosa cells and stromal cells (Seli et al., 2005). On the other hand, we have detected *Epab* mRNA expression at low levels in the granulosa cells as well as oocytes in the follicles at different developmental terms (Ozturk, Sozen, & Demir, 2015). These findings are presented in detailed below. The *Epab* mRNA copy numbers are also analyzed by using quantitative reverse transcription polymerase chain reaction in the oocytes obtained from primary, early antral and antral follicles (Lowther, Favero, Yang, Taylor, & Seli, 2017). The oocytes from preantral follicles have significantly higher *Epab* mRNA copy numbers than those of the oocytes from primary follicles. On the other hand, there are similar numbers in the oocytes recovered from preantral, early antral and antral follicles (Lowther et al., 2017). It is important

to note that since *Epab* is expressed in all follicular stages and can affect the developmental features of the oocytes and granulosa cells, the potential roles of EPAB during oocyte maturation would not be evident in the *Epab* knock out models.

We have examined *Epab* mRNA expression in the postnatal mouse ovaries at different developmental periods (Ozturk et al., 2015). It is at the high levels in the prepubertal ovaries at the ages of 1-, 2-, and 3-week when compared to the later developmental periods including pubertal (4-, 5-, and 6-week old), postpubertal (16- and 18-week old), and aged (52-, 60-, and 72-week old) ovaries (Ozturk et al., 2015). Consistently, when we have counted the number of follicles in these postnatal ovaries, the prepubertal ovaries had a higher total number of follicles than we discovered in the later ovarian stages. The *Epab* mRNA localizes in the cytoplasm of both oocytes and granulosa cells of the follicles from primordial to antral follicles in the prepubertal ovaries (Ozturk et al., 2015). As with the findings in the antral follicles by using RNA in situ hybridization, cumulus cells and MII oocytes obtained from cumulus oocyte complexes (COCs) express the *Epab* transcript (Ozturk et al., 2015). In contrast, Seli et al. (2005) reported that the *Epab* mRNA resides only in the oocytes of growing follicles and, is not detected in the ovarian somatic granulosa cells. The difference between *Epab* expression in the granulosa cells and cumulus cells most likely arises from using different probes for RNA in situ hybridization and technical application discrepancies. This should be evaluated in the future studies that will analyze EPAB expression distribution in the ovarian cells.

In more detailed analyses of the *Epab* mRNA in the oocytes and early embryos, it is found to be expressed in the prophase I (also known as GV) and MII oocytes, and in the 1-cell and 2-cell embryos; this is discussed and compared with *Pabpc1* expression in our study (Ozturk, Yaba-Ucar, Sozen, Mutlu, & Demir, 2016; Figure 3). It is however, at undetectable levels in the 4-cell, 8-cell, and blastocyst stage embryos in mice (Seli et al., 2005). Two *Epab* mRNA isoforms, one at full length and other one lacking exon 11 (encoding a part of PABC domain), are both transcribed in the GV and MII oocytes and in the 1-cell and 2-cell embryos (Seli et al., 2005). On the other hand, Wilkie et al. (2005) unraveled *Epab* expression in the embryos at 14.5 days post coitum. The presence of the *Epab* mRNA expression in the later embryogenesis and its functional importance remains elusive.

Epab plays important roles in stabilizing maternally stored mRNAs and their translational activation in the mouse oocytes and early embryos (Figure 3). Consequently, the absence of *Epab* gene leads to the inability to produce early embryos or mature oocytes in vitro or in vivo, and thus to female infertility (Guzeloglu-Kayisli et al., 2012). The $Epab^{-/-}$ mouse models have been created with the targeted deletion of the exon 2, leading to the formation of a stop codon in the exon 3 (Guzeloglu-Kayisli et al., 2012). Therefore, the truncated EPAB protein, including only a part of the first RNA recognition motif (RRM) is generated, but there are no the remaining RRMs and PABC domain (Guzeloglu-Kayisli et al., 2012). As expected, the loss of EPAB in the mouse oocytes results in not translating the maternally stored mRNAs such as *c-Mos*, *Ccnb1*, and *Dazl* and their

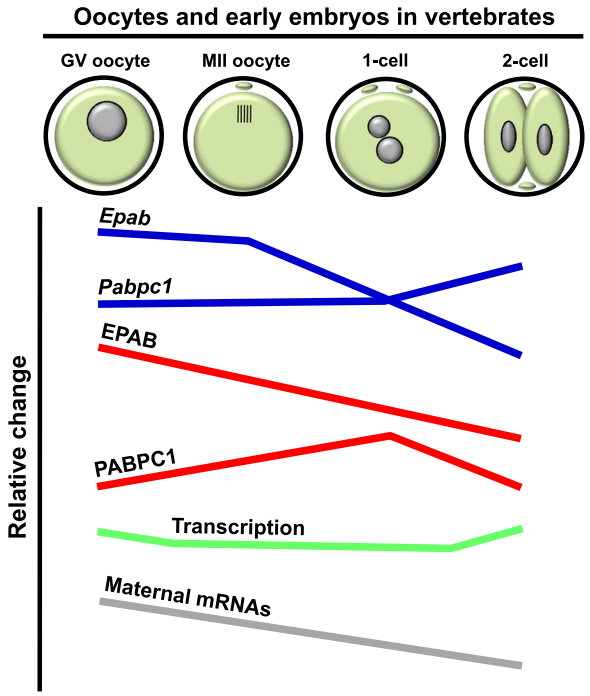


FIGURE 3 The relative changes of embryonic poly(A)-binding protein (EPAB) and poly(A)-binding protein cytoplasmic 1 (PABPC1) expression at mRNA and protein levels, transcription and maternal mRNAs level in mouse oocytes and early embryos. The *Epab* and *Pabpc1* genes exhibit similar and different expression distributions at these stages of early development. The transcriptional activity decreases from GV oocytes to MII oocytes and ceases at the MII oocytes upon meiotic resumption until embryonic genome activation (EGA) at the 2-cell embryos in mice, in which time transcriptional activity rises. Maternal mRNAs are increasingly stored during transcription in the GV oocytes of growing follicles, and their levels begin to gradually decrease from MII oocytes to 2-cell embryos due to undergoing degradation after being used in the translation of the required proteins. Since maternal mRNAs are transcribed and stored during early oogenesis, their levels gradually increase in GV oocytes and then decreases to 2-cell embryos. GV, germinal vesicle; MII, metaphase II [Color figure can be viewed at wileyonlinelibrary.com]

cytoplasmic polyadenylation during in vitro oocyte maturation. In addition, cumulus expansion defect in the late antral follicles, 8-fold decrease in ovulation and remarkably reduced expression of the epidermal growth factor (EGF)-like growth factors mRNAs such as amphiregulin (*Areg*), epiregulin (*Ereg*), and betacellulin (*Btc*), and also the prostaglandin synthase 2 (*Ptgs2*), hyaluronan synthase 2 (*Has2*) and tumor necrosis factor α-induced protein 6 (*Tnfaip6*) genes, which are implicating in cumulus expansion and extracellular matrix synthesis by cumulus cells (Ochsner et al., 2003; Park et al., 2004). The EGF-like factors are required for regulating cumulus expansion (Kawashima et al., 2012). Furthermore, $Epab^{-/-}$ oocytes exhibit defects in the meiotic division and chromosome alignment upon in vitro maturation (Guzeloglu-Kayisli et al., 2012). Also, $Epab^{-/-}$ mouse ovaries possess a 3-fold higher number of secondary follicles when compared to its control counterparts, but there are no differences related to the numbers of other follicle types.

The *Epab*$^{-/-}$ oocytes are, moreover, found to be smaller in size, include peripherally localized germinal vesicle, and exhibit loose connection with surrounding cumulus cells (Lowther & Mehlmann, 2015). There is no surrounded nucleolus (SN) chromatin configuration and no transcriptional suppression in *Epab*$^{-/-}$ oocytes when compared to the controls. Microinjection of *Epab* mRNA into GV oocytes at the preantral follicles consistently provides chromatin reorganization and maturation to metaphase II stage oocytes (Lowther & Mehlmann, 2015), suggesting that the lack of EPAB can be reversed in the preantral follicles following *Epab* mRNA injection. There are also oocyte-somatic cell communication defects in either late antral follicles or COCs due to disrupted gap junctions based on the lack of *Epab* gene (Lowther et al., 2017). In addition, F-actin staining in the transzonal processes (TZPs) is at the lower levels in *Epab*$^{-/-}$ preantral follicles in comparison with the controls, and there is no F-actin staining in the TZPs in *Epab*$^{-/-}$ COCs. The decreased expression of junction protein E-cadherin in *Epab*$^{-/-}$ oocytes (Lowther et al., 2017) may underlie the TZP defects in the preantral follicles and COCs. The lack of the *Epab* gene in the mouse oocytes also causes decreased expression of the genes in the cumulus cells such as lactate dehydrogenase A (*Ldha*), phosphofructokinase (*Pfkp*), and pyruvate kinase muscle isozyme M2 (*Pkm2*), being involved in glycolysis (Lowther et al., 2017). Furthermore, the expression of proapoptotic (Caspase 3 and BAX [BCL2 associated X]) and cell proliferation (proliferating cell nuclear antigen; PCNA) markers is found to be increased in the granulosa cells of the preantral follicles obtained from the *Epab*$^{-/-}$ ovary; however, there are no differences in the early antral and antral follicles (Lowther et al., 2017). Taken together, EPAB seems to play key roles in regulating various types of cellular events such as glycolysis, apoptotic process or more in the granulosa cells and cumulus cells surrounding oocytes in addition to its well-known function related to regulating translational control of the stored mRNAs. Most likely, EPAB fulfills these missions by playing a role in the regulation of various types of mRNAs required for these cellular events. More detailed molecular biological studies are needed, specifically those that attempt to determine the mechanisms by which EPAB contributes to these cellular events during folliculogenesis and oogenesis necessary.

The lack of the *Epab* gene results in defects in both granulosa cell differentiation and cumulus expansion, which is most likely due to altering the EGF signaling between these cells and oocytes (Yang, Lowther, Lalioti, & Seli, 2016). These defects do not derive from changes in the protein expression of growth differentiation factor 9 (GDF9) or bone morphogenetic protein 15 (BMP15) since there are no expressional differences between *Epab*$^{-/-}$ and wild type groups (Yang et al., 2016). Conversely, the decreased phosphorylation levels in the mitogen-activated protein kinase kinase 1/2 (MEK1/2), extracellular signal-regulated kinase 1/2 (ERK1/2), p90 ribosomal S6 kinase (RSK), and EGF receptor proteins occur in response to LH and EGF in the granulosa cells of *Epab*$^{-/-}$ mouse ovaries. The cumulus cells obtained from *Epab*$^{-/-}$ mice cannot respond to the signals originated from wild type oocytes although they express anti-Mullerian hormone (*Amh*), sodium-coupled neutral amino acid transporter 3 (*Slc38a3*), and androgen receptor (*Ar*) mRNAs at the normal levels (Yang et al., 2016), which are the markers of normally differentiated cumulus cells (Diaz, Wigglesworth, & Eppig, 2007). Also, granulosa cells from *Epab*$^{-/-}$ mice are unable to stimulate the ERK pathway and increase the p-EGFR levels in response to LH and EGF factors (Yang et al., 2016). Overall, EPAB regulates the responses of either cumulus cells to EGF, and the ERK pathway or granulosa cells to LH and EGF most likely by regulating expression of the genes playing roles in these processes. The phenotypes observed in the lack of *Epab* most likely originate from the potential defects in the other oocyte-secreted factors, causing abnormalities in follicular development since there is no EPAB expression in the granulosa cells of the follicles at different developmental stages. Using *Epab* knockout mice models in characterizing the potential roles of the EPAB during oocyte maturation seem to be inappropriate because the oogenesis and folliculogenesis processes are commenced at the early developmental periods. Therefore, the lack of EPAB from early stages may affect the results obtained in the COCs collected from mature mice models following superovulation.

In humans, the *EPAB* mRNA is expressed in both ovary and testis tissues as well as in the variety of somatic tissues such as those found in the pancreas, liver, thymus, prostate, kidney and spleen (Guzeloglu-Kayisli et al., 2008). In addition to somatic and gonadal tissues, Sakugawa et al. (2008) detected the *EPAB* mRNA in the unovulated human oocytes and early embryos (1-cell, 2-cell, 4-cell, 6-8-cell, 16-cell, and blastocyst stage embryos). It is at the high levels from 2-cell to 8-cell embryos and decreases toward blastocyst stage (Sakugawa et al., 2008). Although human GV and MII oocytes predominantly express the *EPAB* mRNA, it decreases significantly to low levels in the 8-cell and blastocyst stage embryos (Guzeloglu-Kayisli et al., 2008; Figure 3). It is important to note that human oocytes transcribe only the full-length *EPAB* variant (Guzeloglu-Kayisli et al., 2014). However, the alternatively spliced forms of the *EPAB* transcript are highly expressed in the kidney and liver tissues, and the spliced and full-length *EPAB* variants are in comparable levels in the human ovary where they are composed of either somatic cells or oocytes (Guzeloglu-Kayisli et al., 2014). There are three alternatively spliced *EPAB* transcripts, not having part of exons 8, 9 or 9 and 10 in the human ovary. The lack of the corresponding exons leads to creating premature stop codon, resulting in producing truncated EPAB isoforms (Guzeloglu-Kayisli et al., 2008). Indeed, the spliced form lacking the first 58 bp of exon 8 creates a premature stop codon and leads to partial loss of RRM4 and the absence of the PABC domain. The presence of high-number of exonic splicing enhancer motifs at the first 58 bp of exon 8 may regulate the production of alternatively spliced forms. It is striking that this alternatively spliced *EPAB* mRNA, encoding a truncated protein and appearing following EGA is detected only in the 8-cell and blastocyst stage embryos (Guzeloglu-Kayisli et al., 2014). Though more studies

aiming to determine the functional importance of the alternatively spliced EPAB variants in the human oocytes and early embryos are necessary, they may be undertaken for posttranscriptional regulation of the *EPAB* gene and/or are evolutionary residuals of the *EPAB* mRNA transcribed by the former vertebrates.

5 | THE SPECIFIC ROLES OF EPAB DURING SPERMATOGENESIS

As transcription ceases in the mid-spermiogenesis stage due to highly condensing chromatin in the late spermatids, translational control plays important roles in regulating stored messenger RNPs (mRNPs) whose levels increase from spermatogonia to round spermatids (Steger, 1999; Yanagiya, Delbes, Svitkin, Robaire, & Sonenberg, 2010; Figure 4). The mRNAs undergo polyadenylation in the nucleus and are stored in the mRNPs whose poly(A) tails are approximately 150 adenosines in length during early spermatogenesis. At the later terms of spermatogenesis, the mRNAs in the mRNPs experience deadenylation (to ~30 adenosines in length) so that they are able to associate with ribosomes (Kleene, 1989). EPAB localizes in the cytoplasm of round spermatids and elongating/elongated spermatids as well as in cytoplasmic droplets in the lumen at the later term of spermatogenesis in mice (Uysal & Ozturk, 2019; Figure 4). The specific expression pattern of EPAB suggests that it functions in the translational control of the mRNPs following transcriptional quiescence (Yanagiya et al., 2010). Although the translational complex in the oocytes and spermatids following transcriptional silencing are most likely composed of the same factors, the prominent difference is the poly(A) tail lengths of the mRNAs undergoing translation. This difference may derive from three possible sources. (a) The paternally stored mRNAs are only utilized until the end of spermatogenesis. Therefore, following translation of these mRNAs with short poly(A) tails (having approximately 30 As in length) would be undergone rapid degradation process as is in eukaryotic cells (reviewed in Parker and Song (2004) and Valencia-Sanchez, Liu, Hannon, and Parker (2006)). Conversely, since maternally stored mRNAs are used for synthesizing the required proteins during a long time period, that is, during oocyte maturation, fertilization and early embryogenesis (Sha, Zhang, & Fan, 2019), they experience polyadenylation before translation. (b) The deadenylation and/or polyadenylation mechanisms that take place during later spermatogenesis may differ from that occurring in oocytes. (c) The temporal and spatial levels and cellular localization of the PABPs in the spermatogenic cells may be distinct from those found in the oocytes. Therefore, the poly(A) tails of paternally stored mRNAs cannot be protected as well as those in the maternal mRNAs.

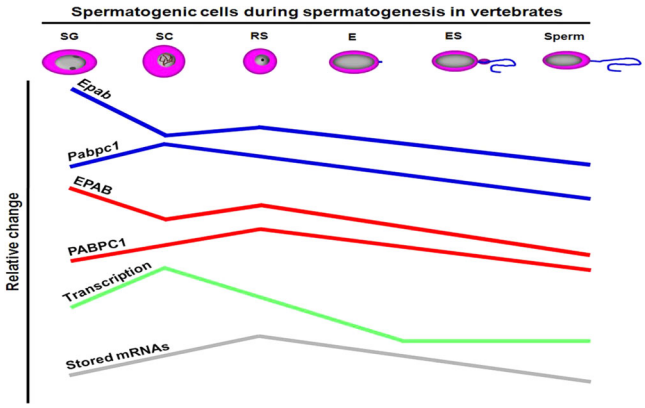


FIGURE 4 The relative changes of embryonic poly(A)-binding protein (EPAB) and poly(A)-binding protein cytoplasmic 1 (PABPC1) expression at mRNA and protein levels, transcription and stored mRNAs in the spermatogenic cells during spermatogenesis in mice. The *Epab* and *Pabpc1* genes exhibit similar mRNA and protein expression in the spermatogenic cells except for spermatogonia. As is well known, paternal mRNAs are transcribed and stored during early spermatogenesis, and their levels gradually decrease after the time point where transcription is ceased at the mid-spermiogenesis. Consistently, stored paternal mRNA levels progressively increase through round spermatids and decrease from round spermatids to sperm cells. E, elongating spermatids; ES, elongated spermatids; RS, round spermatids; SC, spermatocytes; SG, spermatogonia [Color figure can be viewed at wileyonlinelibrary.com]

In the *Xenopus laevis*, the *epab* mRNA is highly expressed in the testis, but it is found at low levels in the kidney and heart tissues (Wilkie et al., 2005). Similarly, *Epab* is transcribed in the mouse testis (Seli et al., 2005; Wilkie et al., 2005); however, there is no expression detected in the somatic tissues of the liver, kidney, heart, and muscle (Seli et al., 2005; Wilkie et al., 2005). Our previous study presents a comprehensive analysis of the temporal and spatial expression of *Epab* in the immature (D6-D32) and mature (D88) mouse testes and in the isolated spermatogenic cells (Ozturk et al., 2012). The *Epab* mRNA levels progressively increase from D6 to D32, and significantly decrease in D88 testis. The enhanced *Epab* expression in the later developmental terms is most likely due to increased expression in the spermatogonial cells and round spermatids (Ozturk et al., 2012). The spermatogonial cells obtained from D6, D8, and D16 testes have *Epab* expression at low levels, but its expression increases significantly in the spermatogonial cells from D32 testis. Analogously, spermatocyte fractions from D32 testes possess higher *Epab* expression than those in the fractions from either D29 or D88 testes. In the mature mouse testes, the round spermatid fractions exhibit significantly higher *Epab* expression when compared to the fractions of the spermatocytes, elongated spermatids, and sperm cells (Ozturk et al., 2012). Since spermatogonial cells could not be isolated at a high level of purity in the mature testes, we have determined higher *Epab* mRNA expression in the spermatogonial cells when compared to other spermatogenic cell types using the RNA in situ hybridization method (Ozturk et al., 2012; Figure 4). Also, we have recently analyzed the cellular and subcellular distributions of EPAB in the mouse testis after producing the EPAB-specific antibody (Uysal & Ozturk, 2019). EPAB expression decreases from spermatogonial cells to spermatocytes, and the increased EPAB expression in the round spermatids decreases toward sperm cells in the mouse testes (Uysal & Ozturk, 2019; Figure 4). The expression pattern of EPAB during oogenesis and spermatogenesis convinced us

that EPAB plays a role in the translational control of both maternally or paternally stored mRNAs following transcriptional silencing.

In spite of displaying an exclusive expression pattern in the mouse testes and isolated spermatogenic cells, *Epab*$^{-/-}$ male mice models are fertile, viable, phenotypically normal in appearance and similar in body weight their to the wild-type and heterozygous counterparts (Ozturk et al., 2014). The *Epab*$^{-/-}$ male mice also have a comparable number of pups and fertility-related parameters such as testis weight, sperm count, morphology and motility, and apoptosis index rate as well as normal spermatogenesis in the seminiferous tubules in comparison with the wild-type and heterozygous breeding males. Although there are no remarkable differences in the expression levels of the mRNAs including *Pum2*, *Dazl*, *Pabpc1*, Moloney sarcoma oncogene (*c-Mos*), and *Paip2*, only *Msy2* expression significantly increases by approximately 50% in the *Epab*$^{-/-}$ mice testes when compared to the controls; however, the MSY2 level is detected at the similar levels in both groups (Ozturk et al., 2014). It has been demonstrated that MSY2, a germ-cell-specific member of the Y-box family of DNA/RNA-binding proteins, plays roles in the transcriptional process and stabilization of the stored maternal and paternal mRNAs (Yang et al., 2005). Probably, the lack of EPAB in the mouse spermatogenic cells may be compensated by other poly(A)-binding proteins such as PABPC1 by the ways of contributing to mRNA stabilization and controlling translation after participating in the translational complex and/or binding to the poly(A) tail (Ozturk et al., 2014). As a result, EPAB seems to be unimportant for male fertility in mice; however, we do not know its functional importance in men's fertility.

In humans, there is only one study in which *EPAB* mRNA expression was analyzed in the testes with non-obstructive azoospermia (Ozturk et al., 2016). In this study, we revealed that there is no difference for *EPAB* expression in the testes from non-obstructive azoospermia groups including hypospermatogenesis, round spermatid arrest, spermatocyte arrest and Sertoli cell-only syndrome groups. Moreover, RNA in situ hybridization application showed that the *EPAB* mRNA localizes in the cytoplasm of both intertubular and germinal epithelial cells in the hypospermatogenesis group. We detected that the *EPAB* expression levels in the isolated spermatocytes and round spermatids fractions significantly decreases from hypospermatogenesis to spermatocyte arrest groups (Ozturk et al., 2016). The spermatocytes fraction from hypospermatogenesis group also has significantly higher *EPAB* expression than that of in the round spermatids fraction (Ozturk et al., 2016).

Although *EPAB* shows exclusive expression patterns in both mouse and human testes, it seems to be dispensable for spermatogenesis based on the findings from *Epab*$^{-/-}$ male mice. More studies are required, specifically those that evaluate the probable relationship between EPAB and other RNA-binding proteins as well as its intracellular interactions with other cytoplasmic proteins and mRNAs in the mouse and human spermatogenic cells before and after transcriptional silencing. On the other hand, the expressional distribution of *EPAB* differs between mouse and human testes because *EPAB* is also expressed in the intertubuler cells in the human testis. We think that this

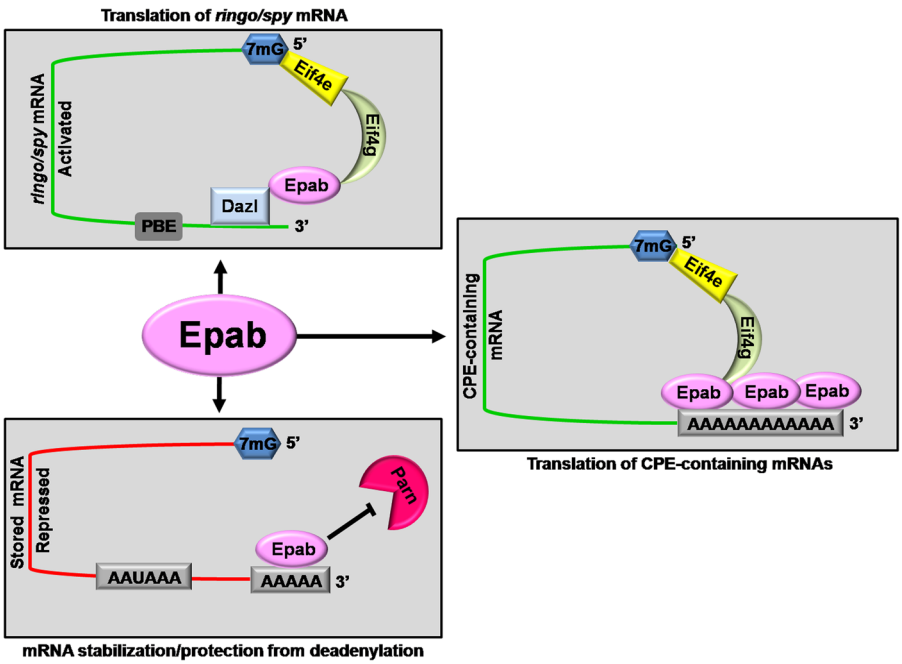


FIGURE 5 The particular functions of embryonic poly(A)-binding protein (Epab) is summarized based on the findings from *Xenopus*. Epab play key roles in: i) Rapid inducer of G2-M in oocytes (*ringo*)/Speedy (*spy*) mRNA translation, ii) translation of cytoplasmic polyadenylation element (CPE)-containing mRNAs, and iii) mRNA stabilization and protection from deadenylation. It is important to note that certain molecules are shown for simplicity in this schematic diagram. 7mG, 7-methyl guanosine; A, adenosine; Eif4e and 4g, eukaryotic initiation factor 4e and 4g; Dazl, deleted in azoospermia-like; Parn, poly(A)-specific ribonuclease; PBE, pumilio binding element [Color figure can be viewed at wileyonlinelibrary.com]

should be analyzed to discover whether it reflects its protein expression in the somatic cells since the *EPAB* gene can generate alternative spliced transcripts.

6 | CONCLUSION

In conclusion, EPAB plays critical roles in preventing default and ARE-mediated deadenylation, stimulating cytoplasmic polyadenylation, and regulating translational activity of the stored paternal and maternal mRNAs during oogenesis, spermatogenesis, fertilization, and early embryo development in vertebrates. The well-known functions of EPAB have been summarized in Figure 5.

A limited number of studies that characterize EPAB functions in these processes have been conducted. The molecular background of the potential roles of EPAB in the mammalian oocytes and granulosa cells during folliculogenesis as well as in early embryos needs to be evaluated in more detail. Although it has been determined that EPAB is required for female fertility in the processes of ovulation and oocyte maturation, and formation of TZPs and so forth it is not known how EPAB carry out the possible intracellular interactions with other cytoplasmic proteins such as signaling molecules. There are still questions about whether EPAB undergoes posttranslational modification(s) at these points to perform necessary functions in a correct and timely manner. This will have to be analyzed in the germline cells and early embryos. More studies are also required to determine the potential relationship of the EPAB protein with the mRNAs and other proteins in the translational regulation during gametogenesis and early embryogenesis. The characterization of uncovered mechanism(s) related to EPAB would contribute to illuminating female infertility development and treatment.

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CONFLICTS OF INTEREST

The author declares that there is no conflict of interest.

ORCID

Saffet Ozturk http://orcid.org/0000-0002-8089-7078

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