Identification and characterization of human embryonic poly(A) binding protein (EPAB)

Ozlem Guzeloglu-Kayisli†, Samuel Pauli†, Habibe Demir, Maria D. Lalioti, Denny Sakkas and Emre Seli1

Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT 06520, USA
†Correspondence address. Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, 300 George street, Suite 770J, New Haven, CT 06511, USA. Fax: +1-203-785-7134; E-mail: emre.seli@yale.edu

Translational silencing that begins with oocyte maturation persists during the initial mitotic divisions of the embryo. Gene expression during this period largely depends on the translational activation of maternal mRNAs by cytoplasmic polyadenylation and requires an embryonic poly(A) binding protein (EPAB). EPAB has been identified in Xenopus and mouse, where it is expressed exclusively in oocytes and early embryos until zygotic genome activation (ZGA) when it is replaced by the somatic cytoplasmic poly(A) binding protein (PABPC1). EPAB plays a central role in the regulation of maternal mRNA activation by preventing deadenylation and promoting translation. In this study, we identified and characterized the human EPAB ortholog. Human EPAB is a 619 amino acid protein with 77% identity and 84% similarity to mouse EPAB. Human mRNA is detected in ovaries, testes and several somatic tissues including pancreas, liver and thymus. Similar to the observations in Xenopus and mouse, human EPAB is expressed exclusively in oocytes and early embryos until zygotic genome activation (ZGA) when it is replaced by the somatic cytoplasmic poly(A) binding protein (PABPC1) following ZGA, which occurs at 4- to 8-cell stage in human. Our findings suggest that the unique translational regulatory pathways that control gene expression during oogenesis and early embryo development may be common between model organisms and humans.

Keywords: embryonic poly(A) binding protein; oocyte; embryo; polyadenylation; translational control

Introduction

In most metazoan, primordial germ cells have an extragonadal origin and migrate to the somatic gonad where they proliferate by mitosis to form oocytes (Matova and Cooley, 2001). Oocytes, in turn, enter meiosis, only to be arrested at the prophase of the first meiotic division (Sagata, 1996; Page and Orr-Weaver, 1997). This first meiotic arrest may last up to a few years in Xenopus and several decades in humans and is characterized by the synthesis and storage of large quantities of dormant mRNA (LaMarca et al., 1973; Rodman and Bachvarova, 1976). When later translated, these maternal mRNAs drive the oocytes’ re-entry into meiosis (Gebauer et al., 1994; Stebbins-Boaz et al., 1996; Mendez et al., 2000) and control the rate of mitosis during the cleavage divisions (Grosisman et al., 2000; Oh et al., 2000; Uto and Sagata, 2000).

The hormonally mediated resumption of meiosis marks the onset of oocyte maturation characterized by drastic changes in both the nuclear and cytoplasmic compartments, and with suppression of transcriptional activity (Davidson, 1986). Thereafter, gene expression during oocyte maturation, fertilization and early embryo development, until the activation of zygotic transcription, is mainly regulated by translational activation of maternally derived mRNAs accumulated in the oocyte during the first meiotic arrest (Gebauer et al., 1994; Stebbins-Boaz et al., 1996; Mendez et al., 2000; Oh et al., 2000).

In Xenopus, after 12 rapid synchronous cleavages generating more than 4000 cells, the mid-blastula transition occurs and is characterized by the activation of zygotic transcription, also called zygotic genome activation (ZGA) (Newport and Kirschner, 1982a,b). In mouse and human, ZGA occurs at the 2-cell, and 4- to 8-cell stages, respectively (Clegg and Piko, 1982; Flach et al., 1982; Braude et al., 1988). Although ZGA occurs earlier in mammals compared with Xenopus, translational regulation of maternally inherited mRNAs appears to utilize similar mechanisms (Stutz et al., 1998; Richter, 1999; Oh et al., 2000).

Translational activation of stored maternal mRNAs is initiated primarily by cytoplasmic extension of their poly(A) tails. Cytoplasmic polyadenylation differs from the nuclear polyadenylation that occurs on virtually every eukaryotic pre-mRNA (Manley, 1995; Wahle, 1995a,b), by its location, regulation and by the fact that it is confined to gametes and embryos; although recent evidence suggests that it also occurs in neurons (Richter and Lorenz, 2002).

Molecular mechanisms regulating cytoplasmic polyadenylation have been studied primarily in mouse (Bachvarova et al., 1985; Vassalli et al., 1989) and Xenopus oocytes (McGrew and Richter, 1989; Paris and Richter, 1990; Paris et al., 1991) and appear to be highly conserved. A cis-acting sequence in the 3′-UTR of the mRNA, the cytoplasmic polyadenylation element (CPE, consensus sequence UUUUAU1-2U), is necessary for cytoplasmic polyadenylation (Bilger et al., 1994; Gebauer et al., 1994; Hake and Richter, 1994; Hake et al., 1998; Stutz et al., 1998). Prior to oocyte maturation,
CPE-containing mRNAs interact with an inhibitory protein called MASKIN, which inhibits the assembly of the translation initiation complex, resulting in translational silencing.

When Xenopus oocytes are stimulated to initiate maturation, CPE-containing mRNAs undergo cytoplasmic polyadenylation (Kim and Richter, 2006; Richter, 2007). However, simple extension of its poly(A) tail is not sufficient to up-regulate translation of an oocyte mRNA, and MASKIN must be displaced. As demonstrated in Xenopus oocytes, displacement of MASKIN and the initiation of translation requires that a poly(A) binding protein becomes associated with the newly elongated poly(A) tail (Cao and Richter, 2002). However, the somatic cytoplasmic poly(A) binding protein, PABPC1, expressed in the cytoplasm of all cells in metazoans is absent in oocytes and early embryonic cells (Stambuk and Moon, 2006); whereas an embryonic poly(A) binding protein (EPAB) is the predominant cytoplasmic poly(A) binding protein during this period (Voeltz et al., 2001; Seli et al., 2005).

Two structurally distinct groups of poly(A) binding proteins have been identified in vertebrates. PABPC1 (Blobel, 1973) [also called PABP1 in human and mouse or PABC in Xenopus (Mangus et al., 2003; Kuhn and Wahle, 2004)], the prototype of the first group, is ~70 kDa and contains four RNA recognition motifs (RRMs) at its N-terminus and a unique C-terminal PABP domain (Mangus et al., 2003; Kuhn and Wahle, 2004). PABPC1 expressed in the cytoplasm of somatic cells in metazoans is implicated in the control of mRNA stability and translation. EPAB, the predominant cytoplasmic poly(A) binding protein in oocytes and early embryos (Voeltz et al., 2001; Seli et al., 2005), an inducible poly(A) binding protein (IPAB or PABC4), described in human T cells (Yang et al., 1995), and a testis-specific poly(A) binding protein identified in human (Feral et al., 2001) and in mouse (Kleine et al., 1994), belong to the same group as PABPC1. PABPN1 [initially called PAB II (Wahle, 1991)] is the prototype of the second group of poly(A) binding proteins. It is smaller (49 kDa) (Wahle et al., 1992), whereas an embryonic poly(A) binding protein (EPAB) is the predominant cytoplasmic poly(A) binding protein during this period (Voeltz et al., 2001; Seli et al., 2005).

The central role in early development suggested for EPAB in the Xenopus model led us to investigate if EPAB is conserved in mammals. We have identified mouse EPab and characterized its expression (Seli et al., 2005). Similar to its Xenopus ortholog, mouse EPab mRNA is expressed in oocytes and early embryos, and becomes undetectable following ZGA, which occurs at the 2-cell stage in mice (Seli et al., 2005). In the current study, we identified and characterized the human EPAB ortholog.

Materials and Methods

Identification and sequencing of the human EPAB ortholog

Nucleotide and protein sequence databases were searched using standard nucleotide–nucleotide BLAST (BLASTN), protein query versus translated database BLAST (TBLASTN) and translated query versus translated database BLAST (TBLASTX) at the National Center for Biotechnology Information Blast Server (http://www.ncbi.nlm.nih.gov/blast). The entire sequence of Xenopus ePAB (Genbank accession number AAK29408) and mouse Epab (Genbank accession number banbkit 1086926) was used.

To determine the human EPAB sequence, RT–PCR fragments created using primers on exons 1, 2, 5, 8, 10, 11, 12 and 14 (Supplementary Table S1) were extracted from the agarose gel using a gel extraction kit (Roche Applied Science, Indianapolis, IN, USA) according to the manufacturer’s protocol. Sequencing was performed by the W.M. Keck Facility at Yale University.

Computerized sequence comparisons and alignments were made using the SEQUENCER program (Gene Codes, Ann Arbor, MI, USA). Pairwise and multiple alignments of the Xenopus, mouse and human EPAB genes and proteins were performed using the MEGALIGN program of the LASERGENE package (DNASTAR, Madison, WI, USA). Gene structure was also determined with GENSCANW (http://genes.mit.edu/cgi-bin/genscan.cgi). The prediction and assignment of the protein structures were performed using PFAM (http://pfam.wustl.edu).

Detection of EPAB mRNA expression in human somatic and gonadal tissues

The expression pattern of human EPAB and PABPC1 mRNA in human somatic and gonadal tissues was determined by PCR using a human multiple tissue cDNA panel (Clontech, Palo Alto, CA, USA). Human EPAB expression was determined using PCR primers on exons 1 and 2 (1P/2R) (Supplementary Table S1), which yield a 260 bp fragment. Amplifications were carried out by 33 cycles of PCR in which the initial 5 min denaturation at 95 °C was followed by a “touch-down” program for 10 cycles of 92 °C for 30 s, 65 °C for 30 s (–1 °C per cycle) and 72 °C for 30 s; and then 23 cycles of 92 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, and final extension at 72 °C for 10 min.

For detection of human PABPC1, PCR primers on exons 8 and 14 (Supplementary Table S1), which produce an 850 bp PCR product, were used. PCR conditions were: 95 °C for 5 min; then 30 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min; finally 72 °C for 10 min. As an internal control, the expression of a housekeeping gene, β-actin, was detected by PCR using primers 5′-GGCCACGGCTGCTTC-3′ and 5′-GTGGCGTACAGGTCT-3′, which yield a 250 bp fragment. All PCR products were separated on 1.5% agarose/TAE gels and visualized by ethidium bromide staining.

Human oocyte and embryo collection

Oocytes and embryos were collected from consenting patients undergoing infertility treatment with in vitro fertilization (IVF) at Yale Fertility Center, New Haven, CT, USA. Institutional Review Board approval was obtained prior to the initiation of the study. Controlled ovarian stimulation was performed using a variety of protocols as previously published (Seli et al., 2007). Patients were monitored per established protocol and were judged to have sufficient follicular maturation when they had two or more follicles with mean diameter 18 mm or greater. Oocytes were collected by transvaginal ultrasound-guided needle aspiration of the follicles under deep conscious sedation. Retrieved oocytes were rinsed, graded and placed in HEPES buffered human tubal fluid (Irvine Scientific, Santa Ana, CA, USA) at 37 °C under 6% CO2 in air. Conventional insemination or intra-cytoplasmic sperm injection was utilized as indicated.

On the day following oocyte retrieval and insemination (Day 1), each oocyte was examined for evidence of fertilization. Those that were found to have two...
pronuclei were placed into individual droplets for culture to the cleavage stage. For culture from Day 1 to 3, 30 μl Scandinavian G1 media (VitroLife) supplemented with 5% human serum albumin (HSA; Irvine Scientific) was used.

An embryo scoring system based on cleavage rate and morphologic assessment was used for the evaluation of embryo quality as previously described (Vegec, 1999; Seli et al., 2007). In those cases where five or more 4-cell Grade 1 or 2 embryos were present on Day 2, the embryos were placed into extended culture media [Scandinavian G2 media (VitroLife) supplemented with 5% human serum albumin (Irvine Scientific)] and cultured until Day 5.

Immature oocytes that contain a germinal vesicle (GV) and mature metaphase II (MII) oocytes that failed to be fertilized after IVF were collected for analysis 24 h after the retrieval. Oocytes that underwent ICSI, or displayed morphologic changes consistent with decreased viability, were not included in the study. Similarly, embryos at 8-cell and blastocyst stage that were donated to research were also collected. The 8-cell embryos were collected on post-fertilization Day 3, and the blastocyst stage embryos were collected on post-fertilization Day 5. Only embryos of highest quality based on morphologic assessment were included in the study. Informed consent in writing was obtained from each couple.

**Reverse transcription of oocytes and embryos**

Total RNA from oocytes or embryos was obtained by using RNAsure Aqueous Microkit (Ambion, Austin, TX, USA) according to the manufacturer’s instructions. RNA was treated for genomic DNA contamination using DNase I (Ambion). Reverse transcription was performed using the RETROscript kit (Ambion) in two steps: first, template RNA was incubated with oligo-d(T) primers at 85°C for 3 min to eliminate any secondary structures, and the reverse transcription reaction was carried out at 42°C for 1 h.

**Quantitative real-time PCR in oocytes and embryos**

EPAB and PABPC1 expression in oocytes and embryos was evaluated by quantitative real-time PCR (qRT-PCR) carried out on an iCycler (Bio-Rad, Hercules, CA, USA). cDNA, prepared from oocytes and embryos as described above, was assayed in triplicate, each reaction using cDNA from the equivalent of two oocytes or embryos. Each experiment was repeated three times.

Reactions were performed using a SYBR Green supermix (Bio-Rad) in the iCycler Q Detection System (Bio-Rad). PCR was performed in an 1× PCR mixture containing 50 mM KCl, 20 mM Tris–HCl, pH 8.4, 0.2 mM each dNTP, 5 μM oligo-dT DNA polymerase, 3 mM MgCl₂, SYBR Green 1, 10 mM KCl, stabilizers and 100 nM forward and reverse primers. The primer sets used for PABPC1 (1F/2R) and EPAB (1F/2R) are given in Supplementary Table S1. RT–PCR from human ovarian cDNA followed by sequencing was performed in order to determine the full-length human EPAB cDNA (see Materials and Methods). The predicted exon/intron boundaries of the EPAB gene were determined by aligning the nucleotide sequence with the human genome, identifying 14 exons on chromosome 20. The open reading frame of the full-length human EPAB is 1857 bp long encoding a 619 amino acid protein, with 77% identity and 84% similarity to mouse EPAB, and with 72% identity and 83% similarity to Xenopus ePAB (Fig. 1B and C, Table I). The highest degree of amino acid similarity was in the protein segment encoded by exons 1–8, followed by exons 11–14 (Fig. 1B). Like other poly(A) binding proteins (Voeltz et al., 2001; Seli et al., 2005), human EPAB contains four RRM domains, each predicted to bind RNA and a C-terminal PABP domain, which putatively functions in protein–protein interactions (Fig. 1D, Table I).

In addition to the full-length form, we identified three alternatively spliced forms lacking part of exons 8, 9 or 10 (two of these are shown in Fig. 2). The form lacking the first 58 bp of exon 8 (data not shown) results in a premature stop codon 6 amino acids downstream, on exon 8. This would encode a truncated protein lacking the fourth RRM and the PABP domain. The alternatively spliced form of human EPAB mRNA without exon 9 encodes a premature stop codon at the end of exon 10. The third alternatively spliced form lacking both exons 9 and 10 encodes a premature stop codon at the end of exon 12. Therefore, these alternatively spliced forms lacking exon 9 alone, or exons 9 and 10, encode truncated proteins without the PABP domain.

**Human EPAB is expressed in somatic and gonadal tissues**

We first evaluated the expression profile of the human EPAB mRNA in human tissues (Fig. 3). Similar to its mouse ortholog, human EPAB mRNA was detected in the ovary and testis (Fig. 3). In addition, the expression of human EPAB mRNA was abundant in pancreas, liver and thymus, whereas the weaker expression was observed in other somatic tissues including brain and lung (Fig. 3). Specific amplification of human EPAB by PCR using cDNA derived from ovary, testis, pancreas, liver, thymus and spleen was confirmed by sequencing. The same tissues were also analyzed for PABPC1, which was ubiquitously present in all tested tissues (Fig. 3).

**EPAB is the predominant poly(A) binding protein in human oocytes**

Next, we evaluated EPAB and PABPC1 mRNA expression during early human development by qRT–PCR. Similar to its mouse ortholog, human EPAB mRNA is expressed in both GV and MII oocytes at significantly higher levels compared to 8-cell and blastocyst stage embryos (P < 0.05; Fig. 4A). The decrease in EPAB mRNA expression observed in 8-cell and blastocyst stage embryos coincided with an increase in PABPC1 mRNA expression (P < 0.05; Fig. 4B). This finding suggests that in human, similar to that observed in Xenopus and mouse, EPAB is replaced by PABPC1 upon ZGA, which occurs at the 4- to 8-cell stage (Braude et al., 1988).

In addition, as the qRT–PCR for both EPAB and PABPC1 were highly efficient (both >90%), our findings suggest that similar to observations in Xenopus and mouse, EPAB is the predominant poly(A) binding protein in human oocytes.

**Discussion**

Here, we report the human ortholog of mouse and Xenopus EPAB. We identify this gene as the human EPAB not only because it exhibits the
highest identity of any human PABP to mouse and Xenopus EPAB, but also because it is located in the human genome at a region consistent with that expected from its mouse ortholog, and its expression pattern during oocyte and early embryo development is consistent with that expected for an embryonic poly(A) binding protein. It is expressed at high levels in both immature (GV) and mature (MII) oocytes while its levels decrease significantly following ZGA.

Human EPAB contains four RRMs at its N-terminus, a characteristic of mouse and Xenopus EPAB as well as PABPC1 from these species (Table I). In Xenopus, the N-terminal regions containing the four RRMs are 82% conserved at the amino acid level between EPAB and PABP1, whereas the C-terminal regions are only 56% identical (Voeltz et al., 2001). Mouse EPAB shows a similar profile with 71% identity to mouse PABP1 in the N-terminal region (amino acids 1–407) and 45% at the C-terminus of the longer splice variant (amino acids 408–608). Human EPAB, similar to that observed in mouse and Xenopus, has 77% identity to human PABPC1 in the N-terminal region (amino acids 1–413) and 53% at the C-terminus (amino acids 414–619). Xenopus, mouse and human EPAB are only 66% identical, due mainly to differences in the C-terminal region. As the C-terminal region likely regulates protein binding, this difference may reflect differences in interacting proteins.

While this manuscript was in preparation, Sakugawa et al. (2008) reported the cloning of the human EPAB gene. However, their report was limited to the identification of the alternatively spliced variant that lacks the beginning of exon 8 and utilizes a cryptic splice site within the exon, resulting in a truncated protein lacking the 4th RRM and the PABP domain. This is one of the splice variants we observed in our study. In addition, we identified two other
Figure 1: continued

Table I. Pattern search and multiple alignment of human, mouse and Xenopus PABPs.

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<th>RRM4 (aa)</th>
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<th>PABP (aa)</th>
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<td>13–84</td>
<td>(5.1 \times 10^{-27})</td>
<td>101–170</td>
<td>(3.7 \times 10^{-27})</td>
<td>193–263</td>
<td>(2.9 \times 10^{-28})</td>
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<td>(1.3 \times 10^{-23})</td>
<td>534–605</td>
<td>(3.6 \times 10^{-39})</td>
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<tr>
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<td>(6.6 \times 10^{-27})</td>
<td>101–170</td>
<td>(4 \times 10^{-23})</td>
<td>193–263</td>
<td>(2.2 \times 10^{-27})</td>
<td>296–365</td>
<td>(4.7 \times 10^{-23})</td>
<td>524–595</td>
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<tr>
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<td>101–170</td>
<td>(2.8 \times 10^{-24})</td>
<td>193–263</td>
<td>(5.7 \times 10^{-26})</td>
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<td>(8.7 \times 10^{-22})</td>
<td>540–611</td>
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<td>101–170</td>
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<td>193–263</td>
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<td>541–612</td>
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alternative splicing variants that lack exon 9 or exons 9 and 10. It is noteworthy that we have previously reported alternative splicing of exons 10 and/or 13 in mouse Epab (Seli et al., 2005, 2008) affecting the same region in the protein. The functional significance, if any, of the splice variants is currently unclear.

Human EPAB mRNA was detectable in multiple somatic tissues, showing a less restricted tissue expression pattern compared with Xenopus or mouse where EPAB expression is confined to gonads (Cosson et al., 2002; Seli et al., 2007). Although the implications of this differential expression are unknown, possible explanations include altered epigenetic suppression in human somatic tissues or a possible role in suppression and activation of pumilio-regulated mRNAs as recently described in Xenopus (Padmanabhan and Richter, 2006). The latter explanation is plausible as PUMILIO-1 shows somatic expression in human (Moore et al., 2003). In addition, cytoplasmic polyadenylation element-binding protein, which is another protein that interacts with Xenopus PUMILIO (Nakahata et al., 2001) and is germ cell specific in Xenopus, shows a much broader somatic expression in human (Welk et al., 2001).

During Xenopus oocyte and early embryo development, EPAB is the predominant poly(A) binding protein expressed, as demonstrated by both western blot analysis and UV-cross-linking to substrates with [α-32P]ATP-labeled poly(A) tails (Voeltz et al., 2001). In the same study, PABPC1 was undetectable in Xenopus until after the early neurula stage (Voeltz et al., 2001). This was consistent with the previous findings of Zelus et al. (1989) and suggested that PABPC1 expression is induced only after ZGA, which occurs at the mid-blastula stage in Xenopus (Newport and Kirschner, 1982a,b).

More recently, Cosson et al. (2002) confirmed EPAB as the predominant PABP during early Xenopus development although, using western blot analysis, they were able to detect a low level of PABPC1 expression prior to ZGA, after which PABPC1 expression dramatically increased.

In mouse and human, ZGA occurs at the 2-cell and 4- to 8-cell stages, respectively (Clegg and Piko, 1982; Flach et al., 1982; Braude et al., 1988). Therefore, PABPC1 mRNA expression is expected to occur at an earlier stage of embryonic development than in Xenopus. We have previously reported low baseline expression of PABPC1 mRNA in mouse oocytes, as well as in 1-, 2- and 4-cell embryos, followed by a significant increase in PABPC1 levels in 8-cell mouse embryos that becomes more pronounced in blastocysts (Seli et al., 2005). In the present study, we demonstrated a similar pattern in human with low levels of PABPC1 expression in PI and MII oocytes, and a significant increase in 8-cell embryos and blastocysts. Thus, although the increase in PABPC1 expression occurs at an earlier developmental stage in mouse and human compared with Xenopus, it closely follows ZGA, suggesting similar regulatory mechanisms in these species.

Although Xenopus (Voeltz et al., 2001), mouse (Seli et al., 2005) and human EPABs are structurally similar to PABPC1, EPABs are the predominant poly(A) binding proteins in oocytes and early embryos, when PABPC1 expression is minimal or absent. Xenopus, mouse and human EPABs are expected to be similar and unique in their contributions to the control of gene expression during oocyte and early embryo development by regulating poly(A) tail length and unmasking/translation of maternal mRNAs. However, when Cao and Richter (2002) tested human PABPC1 and Xenopus EPAB, they found their activities to be equivalent in several in vitro assays assessing the dissociation of MASKIN from eIF4E. It could be that the differences in the C-terminal regions of EPAB versus PABPC1 are...
for EPAB transcription. (RNA was extracted from oocytes and embryos and subjected to reverse trans-
scription lacking exons 10, 13 or both (Seli et al., 2005) and human EPABs as the predomi-
nant poly(A) binding proteins prior to ZGA suggests that the unique transla-
tional regulatory pathways that control gene expression during oogenesis and early embryo development may be common between model organisms and humans. Further investigation of EPAB function is warranted to further delineate the role of EPAB in the regulation of gene expression during early development and to determine whether aberrant EPAB expression and/or function play a role in important reproductive problems such as oocyte aging, reproductive failure, aneuploidy or embryo death.

Figure 4: Quantification of EPAB and PABPC1 mRNA in human oocytes and early embryos using real-time RT–PCR. RNA was extracted from oocytes and embryos and subjected to reverse transcription. (A) qRT–PCR was performed in all samples using specific primers for EPAB, normalized to β-actin expression used as an internal control. (B) qRT–PCR was performed in all samples using specific primers for PABPC1, normalized to β-actin expression used as an internal control. *P < 0.05.

functionally significant only in the context of the oocyte and early embryo development. In mouse, the alternatively spliced forms of Epab lacking exons 10, 13 or both (Seli et al., 2008) may play distinct roles since the C-terminal PABP domain, which is disrupted in the absence of exon 13, mediates critical protein–protein interactions (Kozlov et al., 2001). A similar change in function with alternative splicing is also possible for human EPAB as the exclusion of exon 9 or exons 9 and 10 affect the same region. It is also noteworthy that the relative expression of human EPAB mRNA splicing variants showed tissue-specific patterns (data not shown), suggesting the presence of distinct regulatory mechanisms.

Recently, two groups (Cosson et al., 2004; Good et al., 2004) independently identified a poly(A) binding protein in Xenopus with 50% amino acid identity to Xenopus PABPN1, called EPABP2. Unlike PABPN1, EPABP2 is localized to the cytoplasm. EPABP2 is expressed in Xenopus oocytes and embryos at levels that are constant up to Day 5 of development (tadpoles), but decreases in older embryos and becomes almost undetectable at Day 15. Good et al. (2004) also identified EPABP2 in mouse, where they showed RNA expression in oocytes but not in blastocysts. These observations suggest that EPABP2, like EPAB, may play a role in cytoplasmic polyadenylation of mRNAs. However, it is noteworthy that Xenopus ePABP2 is present for a much longer period than ePAB (Cosson et al., 2004; Good et al., 2004), suggesting that the control of its expression may be independent of ZGA. Moreover, Xenopus ePABP2, unlike Xenopus ePAB, is not able to interact with the cap-binding complex and is therefore unlikely to stimulate translation at the initiation step (Cosson et al., 2004).

As a factor required for both cytoplasmic polyadenylation-dependent and independent pathways that mediate maternal mRNA translational activation (Cao and Richter, 2002; Padmanabhan and Richter, 2006; Vasudevan et al., 2006) and a factor identified in both known protein complexes (cytoplasmic polyadenylation complex and Pumilio-2/DAZL/ePAB complex) that bind and stabilize dormant mRNAs in immature oocytes (Padmanabhan and Richter, 2006; Kim and Richter, 2007), EPAB seems to play a central role in the regulation of gene expression during early development. Identification of mouse (Seli et al., 2005) and human EPABs as the predominant poly(A) binding proteins prior to ZGA suggests that the unique translational regulatory pathways that control gene expression during oogenesis and early embryo development may be common between model organisms and humans. Further investigation of EPAB function is warranted to further delineate the role of EPAB in the regulation of gene expression during early development and to determine whether aberrant EPAB expression and/or function play a role in important reproductive problems such as oocyte aging, reproductive failure, aneuploidy or embryo death.

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