Detection of and differentiation between two ElrA mRNA isoforms found in *Danio rerio*

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Abstract

CPEB proteins are important for proper regulation of polyadenylation and translation of stored mRNAs. Investigations into the roles of these proteins in Danio rerio (zebrafish) suggests a model whereby the sequential translational control of multiple CPEB proteins themselves during zebrafish embryogenesis is required for early patterning of the embryo. The focus of this study is one of these zebrafish CPEB proteins, known as ElrA, whose gene product functions as an RNA-binding protein. Two transcripts for ElrA can be detected in the fertilized egg, which are the result of both alternate splicing and alternate polyadenylation; however, both code for a nearly identical protein, with the exception of an additional 16 amino acids at the N terminus of the protein product of the longer isoform. The shorter transcript is polyadenylated immediately following fertilization, and is therefore presumed to be the transcript loaded onto ribosomes. In order to investigate the role of the second transcript, which has a 3'UTR that is more than 1000 nucleotides longer than that of the shorter transcript, we analyzed the expression levels of both ElrA transcripts via PCR. The presence of both transcripts at the 1-8 cell, 256-cell, and High-cell stages was confirmed through the results, indicating that both maternal transcripts are stable through early development. Further experimentation, including a developmental time course, microinjections of morpholinos targeted to specific ElrA transcripts, and microinjection of RNAs containing portions of each transcript, can shed light on the roles of these respective isoforms.

Introduction

- Experimentation with Xenopus led to the discovery of proteins that bind to cytoplasmic polyadenylation element (CPE) sequences, known as CPE-binding (CPEB) proteins (Hake and Richter, 1994)
- CPEB proteins function in regulation of polyadenylation and translation of stored mRNAs (Hake and Richter, 1994), as well as mediating translational repression in several species (Zhang et al., 2010; Villalba et al., 2011; Weill et al., 2012; Charlesworth et al., 2013)
- In zebrafish, there is an embryonic-type CPEB protein called ElrA, which is a member of the elav family of RNA-binding proteins found in vertebrates (Good, 1995)
- Translation of maternally provided ElrA mRNA is repressed until fertilization. After fertilization, the ElrA protein is then produced, making it available to regulate the translation of additional mRNAs during embryogenesis (O'Connell et al., 2014)
- mRNA sequence data on NCBI suggested the presence of two ElrA isoforms of different sizes: one being 2160 bp long (ElrA short) and the other being 3385 bp long (ElrA long)

Purpose = Analyze the bioinformatic data found for ElrA short and long, as well as detect the presence of these two mRNAs in zebrafish embryos during early embryogenesis

Results

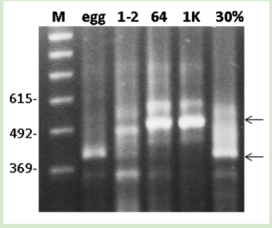
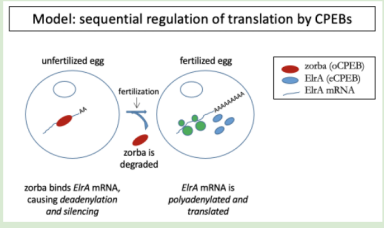


Figure 1: PAT assay for zebrafish ElrA mRNA. Poly(A)+ cDNA was prepared from RNA purified from eggs or the embryonic stages indicated. PCR was performed with an ElrA-specific forward primer that anneals 370 nt upstream of the poly(A) addition site and the anchorT reverse primer. Top arrow, amplification of products with a long poly(A) tail; bottom arrow, amplification of products with a short poly(A) tail. M, 123-bp DNA ladder.



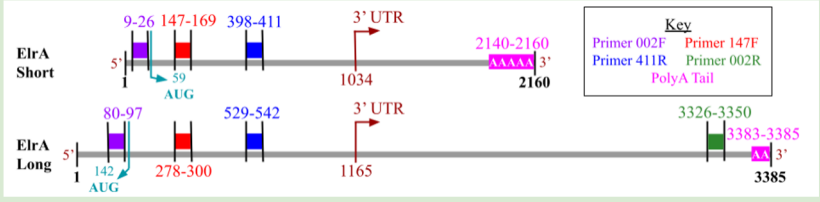


Figure 2. Diagram of the two ElrA maternal transcripts and the primers used in this study. The teal arrow indicates the position of the first AUG that was coded for. The pink indicates the polyA tail position and length. The number of A's listed is not equivalent to the exact number of A's in the polyA tails. PCR was performed with two different ElrA primer sets. ElrA primers 147F and 411R detect the 264bp segment of both ElrA short and ElrA long. ElrA primers 147F and 002R detect the 3,203bp segment of only ElrA long. ElrA primers 002F and 411R detect the doublets of ElrA short and Elra long.

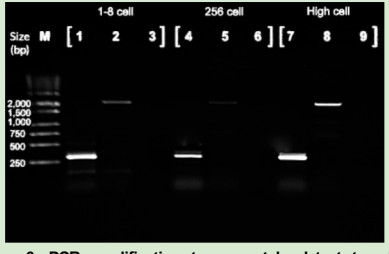


Figure 3. PCR amplification to separately detect two ElrA maternal transcripts. PCR amplification was performed with two different ElrA primer sets. Lane M, 1kb DNA ladder; lanes 1-3, cDNA from 1-8 cell stage, lanes 4-6 cDNA from the 256 cell stage, and lanes 7-9 cDNA from the high stage. The ElrA primers used were 147F and 411R (Lanes 1, 4, and 7) & 147F and 002R (Lanes 2, 5, and 8). Lanes 3, 6, and 9 were negative controls (no primers).

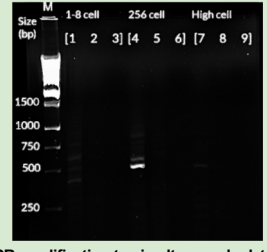


Figure 4. PCR amplification to simultaneously detect two ElrA maternal transcripts. PCR was performed with cDNA prepared from RNA of the 1-8, 256, and high cell stages. Products were separated on an agarose gel. Lane M shows the 1 kB DNA ladder. The combinations of primers corresponding to each lane are 002F and 411 (Lanes 1, 4, 7) & 002F and 002R (Lanes 2, 5, 8). Lanes 3, 6, and 9 are the negative controls (no primers).

Discussion

- Bioinformatic analysis of the two ElrA isoforms revealed that the ElrA long isoform has a longer 5'UTR, an extra 48 nt sequence (coding for 16 amino acids at the beginning of the protein product), and a substantially longer 3'UTR, as compared to ElrA short
- In Figure 3, the bands indicate the presence of the 141F/411R sequence across all stages (Lanes 1, 4, 7), which is found in both ElrA short and ElrA long, suggesting the presence of both ElrA isoforms.
- In Figure 3, the 141F/002R longer sequence (Lanes 2, 5, 8) is also found across all stages. The 002R primer is only found in the 3'UTR of the ElrA long isoform, not ElrA short, suggesting that specifically ElrA long is present in the embryo and can possibly be identified by using the 002R primer sequence.
- In Figure 4, the doublet present in lane 4 (002F/411) indicates the simultaneous presence of both ElrA mRNAs during the 256-cell stage. The varying brightness of the doublet bands suggests that the shorter ElrA isoform is more abundant in the 256-cell stage than is the longer ElrA isoform.

In the future, completing a developmental time course with these ElrA transcripts will provide further information on the expression patterns of the transcripts. Additionally, performing microinjections to either overexpress or knockout the transcripts will be helpful to determine any similarities or differences in the roles of each transcript.

References

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