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PLAUF is a novel *P. lividus* sea urchin RNA-binding protein

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Abstract

Preliminary results have shown that various proteins bind long 3′UTR of the transcript for *Paracentrotus lividus* sea urchin H3.3 histone variant and are probably implicated in mRNA instability. In order to identify these RNA-binding proteins, we screened a λ-ZAPII cDNA expression library prepared from poly(A) mRNA extracted from sea urchin embryos at blastula stage. We isolated a cDNA that codes for a novel RNA-binding protein homologous to rat and human AUF1 family proteins and we refer to it as PLAUF. Proteins present in the whole lysate of the phages expressing PLAUF bound specifically in vitro the 3′UTR of the *H3.3 histone* transcript. Northern blot analysis revealed three *PLAUF* transcripts that are already present in unfertilized eggs; during development their amount increased starting from 4-blastomere embryos and reached the plateau at blastula stage. While the transcription start point was unique, longer 3′UTRs were revealed by 3′RACE approach and further cDNA library screening. Moreover RT-PCR showed the presence of at least one alternative spliced mRNA that codes for a protein with different COOH terminus. The structure of the *PLAUF* gene was determined by screening a *P. lividus* sea urchin genomic library with the *PLAUF* cDNA as probe. Analysis of the positive clones showed that the *PLAUF* gene is split in 10 exons and 9 introns spanning a distance of about 10 kb. Moreover we demonstrated that the exon 9 was alternative spliced during mRNA processing.

Keywords: AUF1; RNA half-life; Gene structure; Alternative splicing

1. Introduction

In eukaryotes, gene expression is regulated at various levels and post-transcriptional regulation is an important control point. At this level, various mechanisms are involved: alternative splicing, that generates an enormous repertoire of functional diversity by producing multiple RNAs and proteins from a single gene (Maniatis and Tasic, 2002); addition of poly(A) tail that provides the mRNA with a binding site for the major class of regulatory factors (PABPs) and promotes translation initiation and termination, recycling of ribosomes and mRNA stability (Mangus et al., 2003); editing (Gerber and Keller, 2001); mRNA localization that targets the proteins on their site of function (Tekotte and Davis, 2002); mRNA decay mediated by defined regions of transcripts that interacted with specific binding proteins (Guhaniyogi and Brewer, 2001).

In addition to the poly(A) tail, several *cis*-acting elements play a role in determination of mRNA half-life: some proteins can bind these elements to protect mRNA from degradation or, on the contrary, to promote degradation attracting degrading enzymes (Bevilacqua et al., 2003). These elements can be localized in the coding region, such as Coding Determinant Region (CDR) of c-myc mRNA (Doyle et al., 1998) or in the 3′UTR such as the AU-Rich Elements (ARE), IRE (Iron Responsive

Abbreviations: AUF1, AU-binding factor 1; ARE, AU-rich element; CDR, coding determinant region; UTR, untranslated region; bp, base pair(s); cDNA, DNA complementary to mRNA; kb, kilobase(s) or 1000 bp; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription and polymerase chain reaction.

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Element) (Erlitzki et al., 2002) and C-Rich Elements (Wein et al., 2003).

Best characterized elements are the AU-rich located in 3′UTR of several labile mRNAs encoding cytokines, oncoproteins and proteins involved in nervous system development (Chung et al., 1996). AREs, originally divided in AUUUA-containing and non AUUUA-containing groups, were then classified on the basis of number and distribution of the AUUUA motifs (Chen and Shyu, 1995). Shaw and Kamen (1986) firstly demonstrated that the AU-rich region of the 3′UTR of the human granulocyte–macrophage-colony-stimulating-factor (GM-CSF) mRNA is a potent destabilizing element. In fact, removal of the AU-rich region increased the half-life of GM-CSF mRNA, while insertion of this sequence into *β-globin* mRNA destabilized the transcript.

A number of *trans*-acting factors that interact with AU-rich sequences have been identified: AU-A, AU-B and AU-C in *lymphokine* mRNAs that shuttle between the nucleus and cytoplasm (Katz et al., 1994), a class of mammalian proteins closely related to the *Drosophila* neuron-specific RNA-binding protein ELAV (*E*mbryonic *L*ethal *A*bnormal *V*ision), including Hel-N1, HuC, HuD and HuR (Ma et al., 1997) and the rat proteins AUF1 (Wilson and Brewer, 1999). AUF1 is a family of four different isoforms (p37, p40, p42 and p45) generated by alternative splicing (Wagner et al., 1998). The fact that AUF1 does not degrade mRNA itself has led to the suggestion that other AUF1 interacting proteins might be involved in the process of selective mRNA degradation. ARE-mRNA degradation involves ubiquitin–proteasome activity, and one or more AUF1 proteins are thought to be ubiquitinated (Laroia and Schneider, 2002). The AUF1 proteins are largely nuclear but they are found also in the cytoplasm and are thought to undergo nucleocytoplasmic shuttling (Sarkar et al., 2003).

Several RNA-binding proteins have a pivotal role in stabilizing, packaging and targeting mRNA, by directing the assembly of multiprotein complexes on primary transcripts, mature mRNAs, and stable ribonucleoprotein components of the RNA processing machinery. The different functions performed by these proteins depend on their dual ability to recognize RNA and to interact with other proteins, often utilizing specialized auxiliary domains (McCarthy and Kollmus, 1995). These proteins are characterized by several conserved RNA-binding motifs such as RRM, CDS and RGG box (Burd and Dreyfuss, 1994). The most common RNA-binding motif is the *RNA Recognition Motif* (RRM) constituted by 90–100 amino acids forming four β-strands and two α-helixes (Burd and Dreyfuss, 1994). These motifs are present in multiple copies in each protein: ELAV-like proteins are characterized by three RRMs, the first and the second of which are responsible of specific ARE-binding and the third is responsible of the poly(A) tail binding (Kiledjian et al., 1997). AUF1 proteins instead are characterized by two RRMs and a RGG box all responsible of specific mRNA binding.

cDNA coding for H3.3 histone replacement variant has been previously isolated from a library of sea urchin *Paracentrotus lividus* embryo (Fucci et al., 1994). The transcript is characterized by 1000-nt-long 3′UTR with dispersed AUUUA/AUUUG and stretches of U, suggesting a possible role of this long 3′UTR in defining the half-life of the molecule. Preliminary results have shown that various proteins binding specifically *H3.3 histone* 3′UTR are present in the whole extracts of embryos. In order to identify such proteins, we screened a cDNA expression library and we isolated a full-length cDNA clone coding for a RNA-binding protein named PLAUF. Then we determined its expression pattern during sea urchin development and the gene structure. Finally we have identified a potential alternative splicing product that gives rise to a shorter protein and three polyadenylation signals that led to three mRNAs containing 3′UTRs of different lengths.

2. Materials and method

2.1. Screening of cDNA and genomic libraries

A cDNA expression library in λ-ZAPII (Stratagene), prepared from poly(A) mRNA extracted from *P. lividus* embryos at blastula stage, was screened following Sägesser et al. (1997) method using as probe the *H3.3 histone* 3′UTR (from nucleotide position 776 to 1562) labelled with [α-^{32}P]UTP by "run off" experiment according to Sambrook et al. (1989). In the primary screening, phages in the liquid lysate were tested for binding to ^{32}P-label 3′UTR of the *H3.3 histone* transcript. After four screenings, we identified a single phage plaque that expressed a protein putatively binding the *H3.3* 3′UTR. The cDNA was rescued into pBlueScript SK(−) vector.

In order to find the other *PLAUF* cDNAs, a new library in λ-ZAPII containing pBK-CMV vector was screened. Approximately 15×10^4 recombinant phages were transferred onto nylon filters (BioBlot-NC) that were hybridized at 65 °C, washed at high stringency and then exposed to X-ray film (Fuji) as described (Fucci et al., 1994). The probe was produced by PCR using the *PLAUF* cDNA as template with PL1 (5′-GAAGAAAAAGATCTTCATCGG-3′) as forward primer and PL2 (5′-ACACGTCTTTAGC-GAGTG-3′) as reverse primer.

The same technique was used to screen a genomic library; DNA was isolated from sperm of individual *P. lividus* sea urchin, partially digested with *Eco*RI and cloned into λ-DASHII vector (Stratagene) using standard procedures (Sambrook et al., 1989). For screening the library, a 739-bp ^{32}P-labelled fragments of the *PLAUF* cDNA was used as probe; it contains the entire 5′UTR and part of the coding region. This fragment was produced by PCR using the *PLAUF* cDNA as template with PL5 (5′-CCTTGAC-GAACATCCCGGAGGAA-3′) as forward primer and PL6 (5′-CCTCTTCCGCCCATCATACC-3′) as reverse primer.