Effect of truncated mutants of poly-A binding protein (PABP) to termination of translation *in vitro*

Protein biothynthesis lab

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Introduction

Poly-A binding protein (PABP) protects the poly-A tails of eukaryotic mRNA from degradation and allows the formation of closed-loop structure of mRNA. In addition it was found that PABP specifically binds eukaryotic translation termination factor eRF3. However, the function of this interaction remains obscure. PABP consists of 2 functional regions: N-terminal region, that binds poly A tail and the C-terminal region that interacts with eRF3. These two domains are connected by linker region. N-terminal domain contains four motifs: RRM1,2 specifically bind poly-A sequence; RRM3,4 nonspecifically bind any RNA (fig. 1).

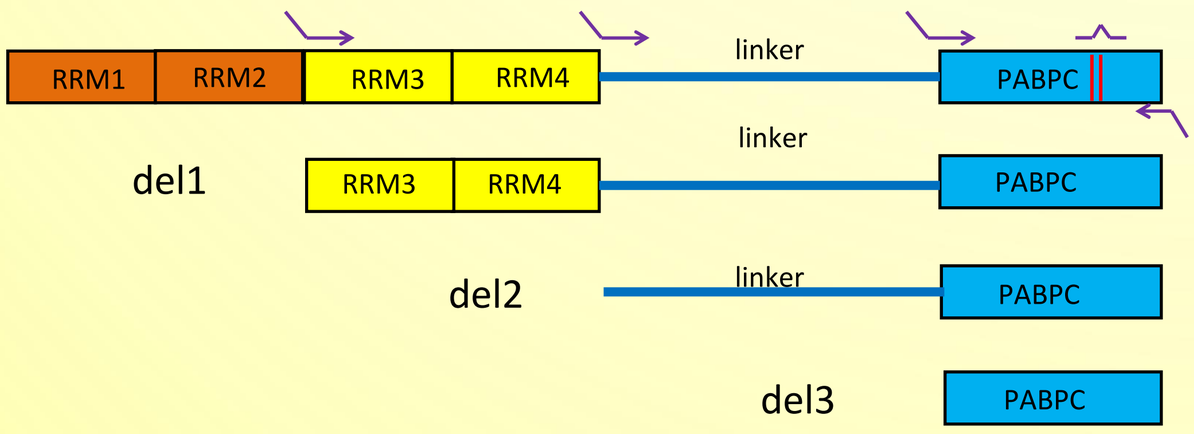


Figure 1 Scheme of structure PABP and its deletion mutants

Purpose

Study of the contribution of the functional domains of PABP in eukaryotic translation termination *in vitro*.

Experimental procedures

1.Preparation of PCR of megaprimer with point mutations to remove the internal NdeI and XhoI sites of restriction. Human PABP gene that was cloned into a vector pET3b used as a matrix.

2.Creating truncated mutants with using the megaprimer. Creating del1, del2, del3 trumcated mutants (fig. 1).

3.Cloning this PCR fragments to pET15b vector by sites NdeI – XhoI (fig. 2).

4.Transformation with prepared constructions of strain of E.coli TOP10F and PCR-screening of the colonies.

5. Expression of mutant proteins in BL21 pUBS.
6. Extraction of the proteins from E.coli and following purification of them in Ni-NTA sepharose. Analysis of protein fractions electrophoresis in PAAG.
7. Studying effect of mutant proteins to termination of translation in reconstituted eucariotic translation system (RETS) by toe-print assay (fig. 3).

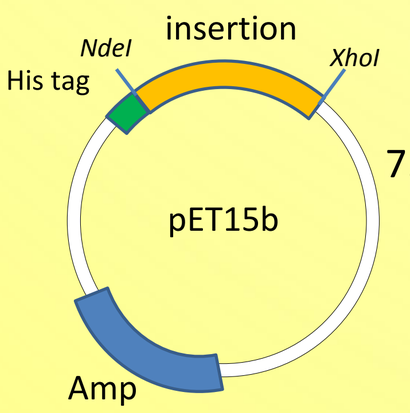


Fig. 2 Scheme of plasmid

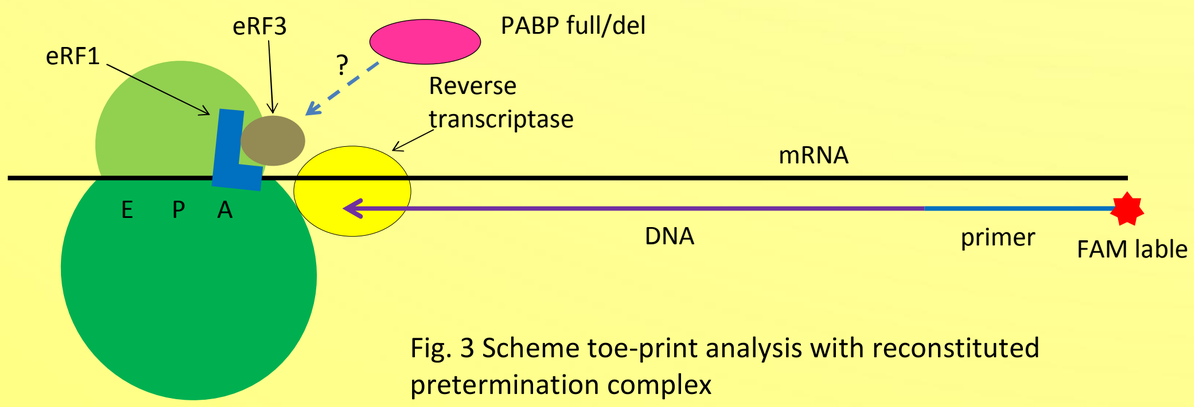


Fig. 3 Scheme toe-print analysis with reconstituted pretermination complex

Results

Getting of gene constitutings

We synthesed PCR fragments with corresponded del1, del2, del3 deletions with using the megaprimer. (Fig. 4).

We cloned pET15b PCR fragments. We do PCR-screening of grown colonies with using 2 sets of primers. At least 3 colonies with insertion of correct size were found by results of PCR-screening.

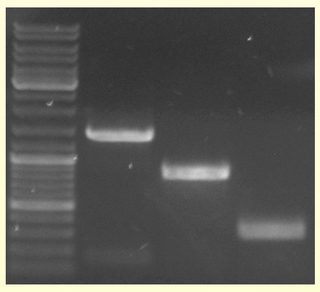


Fig.4 PCR fragments

We extracted plasmids from positive clones and checked them by electrophoresis (Fig. 5).

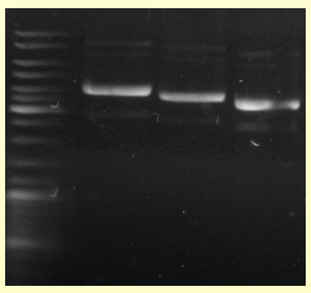


Fig.5 pET15b with insertions

Protein expression

We expressed protein in BL21 pUBS strain and purified them by affinity column (Fig. 6).

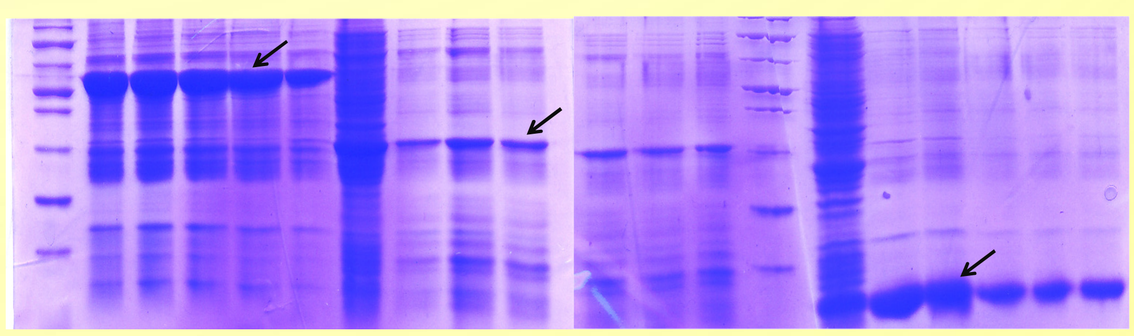
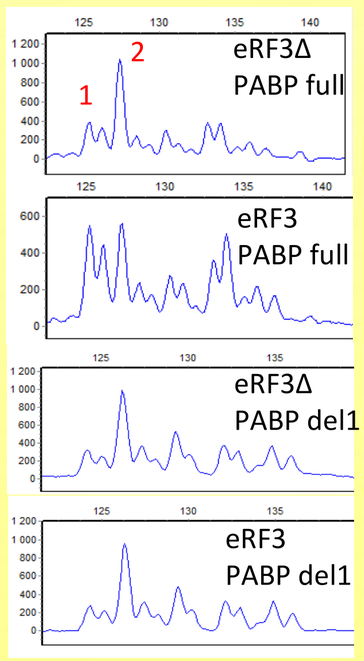


Fig. 6 PAAG electrophoresis of extracted mutants .
1-5 – PABP del1, 6-12 – PABP del2, 13-18 – PABP del3

Toe-print in reconstituted system of translation

Using toe-print we found that truncated PABPs have no effect on termination of translation. We conclude full length PABP is necessary for stimulation of termination (fig. 7). Truncated eRF3Δ that can't bind PABP was used as a control for specific action of PABP.



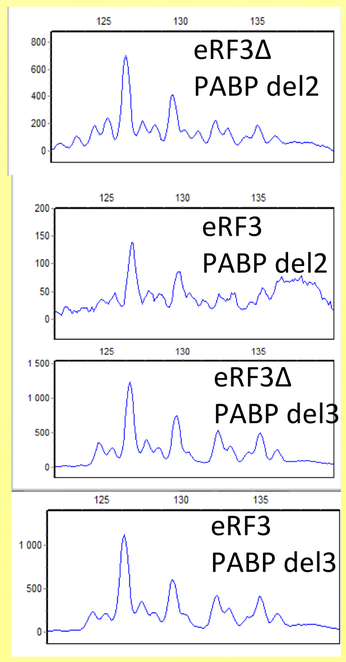


Fig 7. Toe-print assay of termination in addition of PABP.
Peaks correspond ribosome position at mRNA. Peak 1 corresponds termination complex (TC), peak 2 – pretermination complex (preTC).

Conclusions:

1.Truncated mutants of PABP were obtained

2.Only full length PABP stimulate termination, truncated PABPs have no effect.