Heterologous Expression of a Human Cap-Binding Protein eIF4E in *Escherichia coli*

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ABSTRACT: A crucial step in the initiation of cap dependent translation of eukaryotic mRNA is the interaction of the mRNA cap-binding protein, eIF4E, with eIF4G, two integral components of the mRNA cap-binding complex. The present study was aimed to express human eIF4E at high level in *E. coli*. For high-level expression, the *E. coli* cells were first transformed with an expression vector that contains the cloned gene, human eIF4E. Following transformation, the recombinant plasmid was purified and stepwise digestion of the recombinant plasmid revealed that the plasmid was harboring an insert, equal to the length of the eIF4E gene. As the vector contained a heat-inducible promoter, high-level expression of human eIF4E was carried out by heat induction at 42ºC. Expression of recombinant protein was observed by comparing the cell extracts collected before and after heat induction and their subsequent analysis on a denatured polyacrylamide gel. The appearance of a 24-kDa protein following induction of transformed cells confirmed the expression of the cloned gene, human eIF4E.

Key words: Translation initiation, cap-binding protein, eIF4E

INTRODUCTION

Protein biosynthesis is a central biological process in all living cells. It takes place on the ribosomes where the genetic information, transcribed into mRNA is converted into protein in a process called 'translation'. The initiation stage of translation, in which at least 25 polypeptides interact together along with the two ribosomal subunits and therefore offers not only the most complex step of translation, but also the most significant rate-controlling step and the most frequent target for regulation of translation.[1-3]

In addition to the coding sequence, eukaryotic mRNAs possess post transcriptionally modified noncoding sequences; a cap structure at the 5' end and a 3' poly(A) tail that determine the translational efficiency. The cap structure is an inverted 7-methyl guanylate residue added co-transcriptionally to the first transcribed residue via a triphosphate bridge at the 5' end of mRNA. Promoting initiation of translation is one of the key functions of this molecule.[4]

In eukaryotes, ~95% of cellular translation is cap dependent. Translation initiation consists of several steps and is catalysed by a number of trans-acting factors referred to as eukaryotic initiation factors (eIFs). The recruitment of ribosomes to eukaryotic mRNAs is catalyzed by the factors of the family of eIF4 group which includes the following candidates: eIF4A, eIF4B, eIF4G, and eIF4E and requires the energy provided by hydrolysis of ATP.[5,6] The eIF4A, eIF4E and eIF4G form a stable complex termed eIF4F. In the most general mechanism for ribosome binding to mRNA, eIF4F binds directly to the cap structure via eIF4E. The eIF4E is a 24-kDa cap

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binding protein that recognizes the cap structure specifically and thereby helps the protein synthetic machinery to isolate mRNA for translation.

Assembly of the eIF4F complex is inhibited by a family of repressors termed the eIF4E-binding proteins (4E-BPs). The 4E-BPs (4E-BP1, 4E-BP2 and 4E-BP3) are small heat-stable proteins which inhibit cap-dependent translation.[7-9] Binding of the 4E-BPs to eIF4E is regulated by phosphorylation: hypophosphorylated 4E-BPs interact strongly with eIF4E, whereas the hyperphosphorylated forms bind weakly.[6]

In order to gain deeper understanding of the function of eIF4E, we aimed to express the protein at high level following heat induction at 42^{o}C as preliminary steps for protein purification. *E. coli* was chosen as the host because prokaryotes do not contain endogenous cap-binding protein that might form a complex with the over expressed protein.

MATERIALS AND METHODS

Bacterial strain. *Escherichia coli* DH5α was used to make cells competent for bacterial transformation.

The plasmid. The *E. coli* expression vector pCYTEXP3[9] was used for gene cloning. It was made recombinant by inserting human cDNA encoding eIF4E into its *Nde*I and *Bam*HI restriction sites. The subcloning was done by Dr. M. M. Karim of University of Manchester Institute of Science and Technology, Manchester, UK.

Preparation of competent cells. 100 ml of LB broth was inoculated with 0.01 volume of a fresh overnight culture of the desired *E. coli* strain and grown at 37°C to an OD_{600} of 0.5 to 0.6. Cells were chilled on ice for 10-15 min. They were harvested by centrifugation (750-1000 ×g, 12-15 min, 4°C). The pelleted cells were resuspended in 0.5 volume of ice-cold 0.1 M $CaCl_2$ solution, kept on ice for 30 min, harvested as before and then resuspended in 0.25 culture volume of $CaCl_2$. Cells were stored on ice for a minimum of 2 hours (preferably overnight, as the cells are best competent in the following day of preparation). For storage, glycerol was added to attain a final concentration of 20%.

Bacterial transformation. 100 µl of the competent cells were mixed with DNA solution (1 µl) incubated on ice for 20-30 min. The cells were heat shocked at 42°C for 1 min and chilled immediately on ice for 2 min. They were allowed to recover by addition of 400 µl of LB and incubation for 45 min at 30°C because the transformed plasmid contained a heat inducible promoter. The transformed cells were plated onto selective LB agar containing ampicillin (100 µg/ml) antibiotic.

Restriction Endonuclease digestion of DNA. Plasmid DNA was purified by standard procedures as described in Sambrook *et al.*[10] This was digested with the restriction enzymes *Nde*I and *Bam*HI. The digested DNA fragments were separated by agarose gel electrophoresis.

Expression of the recombinant protein following heat induction. A 10-ml medium of LB with added ampicillin (100 µg/ml) was inoculated with overnight grown recombinant clones of bacterial culture so that the initial OD measured at 0.1. The medium was incubated at 30°C in an orbital shaker rotating at 200 rpm till it reached an OD of 0.6 to 0.8. Then it was transferred in a pre-heated water-bath shaker, set at 42°C for heat induction and was rotated at 200 rpm for four hours. In each hour interval of heat induction, 2 ml of the culture broth was taken after measuring their respective optical densities. All the aliquots, collected at different time intervals were spun down to pellet the bacterial cells that was resuspended in the sample buffer. This was added according to the calculation: 50 µl SDS-PAGE sample buffer / ml of sample / OD 1.0.

Heating at 95^{o}C for 10 to 15 min lysed the resulting cell suspension. The lysate was spun down for 20 min at top speed and the supernatant was transferred in a clean microfuge tube. It now contained the denatured proteins of the cell. A 10 µl aliquot of protein sample was resolved by electrophoresis on a denaturing polyacrylamide gel to observe the expression of the recombinant protein.

RESULTS AND DISCUSSION

Bacterial transformation. The competent cells were transformed with the vector pCYTEXP3 containing the human eIF4E gene. After 24 hour incubation at 30ºC the number of colonies on the LB ampicillin media was counted. As a negative control no DNA was added to the competent cells, hence no growth was observed indicating the success of transformation (Figure 1).

Restriction analysis of the purified plasmid. Plasmid DNA was purified from transformed cells and undigested DNA was checked on an agarose gel for its purity (Figure 2, lane 1). This was digested with restriction enzyme *Nde*I and *Bam*HI. Once the Restriction digestion is completed, agarose gel electrophoresis was performed. A single digestion with *Bam*HI produced only a single fragment of 5 kb, the combined size of the vector plus insert (lane 2). Digestion with both *Bam*HI and *Nde*I produced two fragments, the linearized empty vector, 4 kb in size and another was 650 bp long fragment (lane 3). The eIF4E gene is 651 bp long. The stepwise digestion of the recombinant plasmid, therefore, reveals that the supplied plasmid is harboring an insert, which is equal to the length of the eIF4E gene.

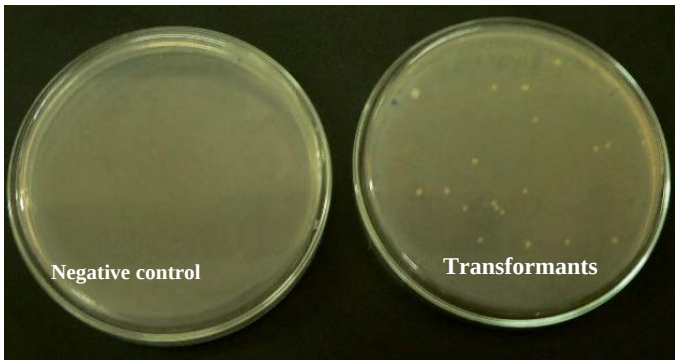

Figure 1. Ampicillin plate showing transformants.

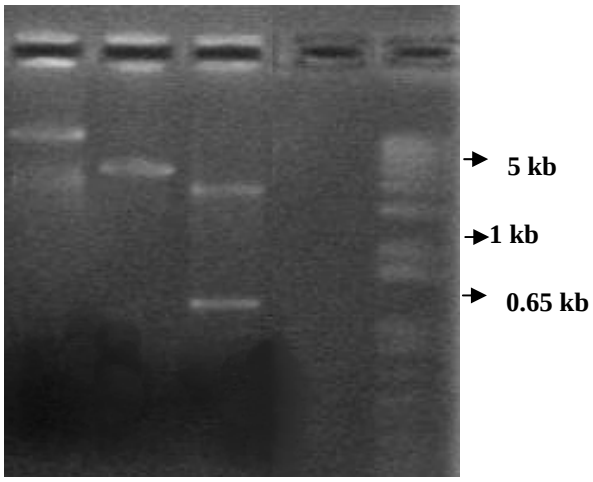

Figure 2. Agarose gel electrophoresis of digested plasmid DNA. Lane 1 shows undigested plasmid DNA; lane 2 shows plasmid DNA digested with *Bam*HI only and lane 3 shows plasmid DNA digested with both *Bam*HI and *Nde*I. The DNA markers (lane 4) are indicated on the right-hand side of the gel.

Expression of human eIF4E. After confirming the presence of the eIF4E gene, a single colony from the transformed population of *E.* coli DH5α cells was inoculated in a tube containing 5 ml LB with ampicillin (100 µg/ml) and incubated overnight at 30ºC. In the following morning OD of overnight culture was set at 0.1 in a 100 ml LB with ampicillin (100 µg/ml) medium and incubated at 30ºC in an

orbital shaker with 200 rpm. When cells reached an absorbance of 0.5, high-level expression of human eIF4E was carried out by heat induction at 42ºC. Figure 3 is a graphical representation of cell count in each hour interval, measured by taking absorbance at 600 nm following heat induction. A maximum population was recorded in third hour post-induction.

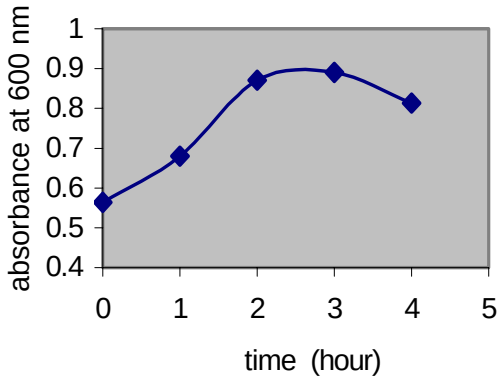

Figure 3. Graphical representation of the absorbance versus time.

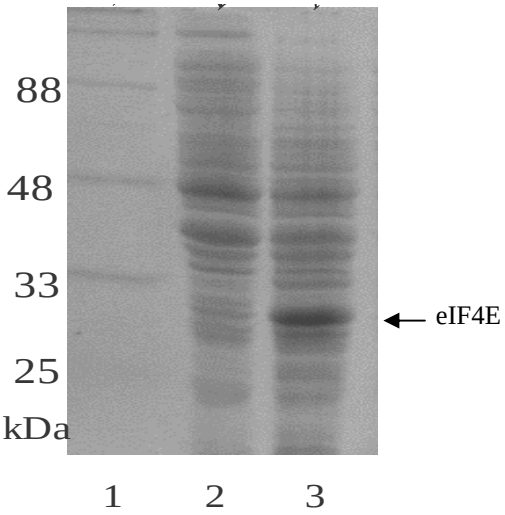

Figure 4. Expression of human eIF4E. A Coomassie blue-stained SDS-15% polyacrylamide gel show the uninduced (lane 2) and induced (lane 3) cell lysates at the end of 3 hours of heat induction at 42ºC. The molecular weight markers (lane 1) are shown on the left-hand side of the gel. Position of eIF4E is indicated to the right.

Cells of third hour post induction were collected and spun down to pellet the cells and were resuspended in the sample buffer. Cell lysate was prepared by heating and the denatured proteins of the cell were collected. The protein samples were resolved by electrophoresis on a denaturing polyacrylamide gel to observe the expression of recombinant protein. Figure 4 shows an analysis of cell lysates, collected before and after 3 hours of heat induction at 42ºC, on a SDS-15% polyacrylamide gel. Comparison between lanes 2 and 3 reveals that a protein of 24-kDa size was conditionally expressed upon induction with heat at 42ºC. This confirms the over expression of human eIF4E protein in *E. coli*. Currently, we aim to purify the protein using 7-methyl GTP-Sepharose as cap analogue resin for the affinity chromatography.

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