Cell-free synthesis of recombinant proteins from PCR-amplified genes at a comparable productivity to that of plasmid-based reactions

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Abstract

The functional stability of mRNA is one of the crucial factors affecting the efficiency of cell-free protein synthesis. The importance of the stability of mRNA in the prolonged synthesis of protein molecules becomes even greater when the cell-free protein synthesis is directed by PCR-amplified DNAs, because the linear DNAs are rapidly degraded by the endogenous nucleases and, thus, the amount of mRNA transcribed is limited. With the aim of developing a highly efficient cell-free protein synthesis system directed by PCR products, in this study, we describe a systematic approach to enhance the stability of mRNA in cell-free extracts. First, exonuclease-mediated degradation was substantially reduced by introducing a stem-loop structure at the 3′-end of the mRNA. The endonucleolytic cleavage of the mRNA was minimized by using an S30 extract prepared from an Escherichia coli strain that is deficient in a major endonuclease (RNase E). Taken together, through the retardation of the endonucleolytic and exonucleolytic degradations of the mRNA molecules, the level of protein expression from the PCR-amplified DNA templates becomes comparable to that of conventional plasmid-based reactions. The enhanced productivity of the PCR-based cell-free protein synthesis enables the high-throughput generation of protein molecules required for many post-genomic applications.

References

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