Effect of energy source on the efficiency of translational termination during cell-free protein synthesis

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Abstract

We studied how the fidelity of translation termination is affected by the method of ATP regeneration during cell-free protein synthesis. During the in vivo expression of hEPO, whose termination is directed by the UGA codon, we found that substantial proportions of the translational products showed a larger molecular weight than expected. Similar results were obtained in a cell-free synthesis reaction using phosphoenol pyruvate (PEP) or 3-phosphoglycerate (3PG) for ATP regeneration. However, when the energy source was switched to creatine phosphate (CP), the readthrough of the UGA codon was completely repressed and only the target protein of the correct size was expressed in a high yield. To the best of our knowledge, this is the first report describing the relationship between the regeneration of nucleotide triphosphates and protein readthrough, and we also believe that the discovery would pave the way to the selective and efficient expression of target proteins in cell-free protein synthesis systems.

References

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