Characterization of the EF-IV (LepA) influence on mRNA translation by in-silico cell-free protein expression

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The LepA is a high conserved protein and an essential elongation factor for ribosome function. Its dominant role is to facilitate the back-translocation of the ribosome when defective translocation occurs during mRNA translation. We demonstrate the results of the in-silico characterization of the error correction role of EF-4 as well as its catalytic and toxic effects in virtual cell-free protein expression systems. The EF-4 role in ribosome back-translocation is described using computer simulation and validated by the experimental data. The results indicate that catalytic effects of the EF-4 originate outside of the translocation mechanism and must be introduced separately from the translocation model. The error correction and toxic effects of this elongation factor clearly reproduce the experimental evidence and confirm the back-translocation origin of this effect. These results together with the calibrated simulation allow for quantification of the catalytic and toxic effects of EF-4. In order to study the dynamics of the protein synthesis under influence of the elongation factor IV (LepA) we employed calibrated and validated computational approach to cell-free protein expression systems. The calibration procedure is demonstrated by the simulation of the in-vitro 5 hours of Luciferase production within the environment equivalent to Rapid Translation System RTS 100 by Roche. In addition to calibration, the model is validated by the simulation of the Edeine antibiotic effect. The integration of the hybrid model of mRNA translation with the model of ribosome translocation is presented. The modeling technique employs the combination of the 3D cellular automata and agent-based simulation. Stochastic nature of the biochemical processes orchestrated by the ribosome are represented by the set of Markov chain that reflect the distinct treatment of the cognate, near- and non-cognate tRNA by the ribosome. The flexibility and adaptability of the presented model combined with with computer simulation is illustrated by the ability to reproduce a number of behaviors observed in the in-vitro experimentation. The application of the resulting computational system is illustrated by the virtualization of the cell-free protein expression kits.