The influence of tRNA located at the P-site on the turnover of EF-TuůGTP on ribosomes

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The turnover of EF-Tu.GTP on poly-U programmed ribosomes was measured both in the presence and in the absence of N-acetylated Phe-tRNA(Phe) at the P-site. The reaction was uncoupled from protein synthesis by omitting Phe-tRNA(Phe) at the A-site. In this reaction, the ribosome can be considered as an enzyme catalysing the transition of EF-Tu.GTP to EF-Tu.GDP. A constant EF-Tu.GTP concentration is maintained by regenerating GDP to GTP at the expense of phosphoenolpyruvate by pyruvate kinase. The rate constants are determined using a procedure which corrects for the reduction in specific activity of GTP due to regeneration of the nucleotide. Ribosomes with an occupied P-site are more efficient in stimulating the GTPase of EF-Tu.GTP than ribosomes with an empty P-site. The data suggest that this is mainly caused by an increased affinity of EF-Tu.GTP for ribosomes with a filled P-site rather than by an enhanced reactivity of the GTPase centre.

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