Abstract

Efficient ribosomal protein synthesis is dependent on *cis*-acting elements in the 5′ untranslated region (*UTR*) of mRNAs. Between prokaryotes and eukaryotes, the sequence and location of these elements differ to the extent of not being functionally interchangeable. We explored the possibility of constructing bifunctional *UTR*s that could direct translation in both prokaryotes and eukaryotes. A variant of a *UTR* from *ner* of phage Mu (*ner*-ACC) enhanced protein synthesis in a rabbit reticulocyte lysate, and it was compared to a *lacZ*-CTA, containing the λ *cro* RBS and the *Escherichia coli lacZ* spacer. Several mutants in the −3 to −1 regions of both *lacZ*-CTA and *ner*-ACC were tested in rabbit reticulocyte lysate and *E. coli* to select *UTR*s that were optimized simultaneously for both biological kingdoms. The *lacZ*-ATC proved 217-fold more effective than *ner*-ACC in this cross-species ability to enhance translation. The *lacZ*-ACC and *ner*-ATC were 83- and 78-fold, respectively, better than *ner*-ACC. We conclude that short *UTR*s (12–15 nt in length) can be fine-tuned in the −9 to −1 regions to enhance protein synthesis concurrently in prokaryotes and eukaryotes. In related studies, we show that nt at the −3 to −1 region of mRNAs exert an enormous impact on synthesis of proteins in *E. coli*.