Blocking IF3 N-terminal domain delays 30S initiation complex formation in bacterial protein synthesis

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Bacterial translation initiation factor IF3 has an essential role in 30S initiation complex (IC) formation. In the initiation phase, IF3 binds to the 30S ribosomal subunit rapidly and modulates the fidelity and speed of the codon-anticodon interaction between the mRNA and initiator tRNA. In canonical conditions, most of the IF3 functions are granted to the C-terminal domain, while the N terminal domain (IF3N) functions remain in debate. Here, we developed an aptamer for IF3N using the Systematic Evolution of Ligands by EXponential enrichment (SELEX) methodology and utilized biophysical methods to study aptamer-mediated blocking of IF3N during 30S IC assembly. Five potential aptamers were identified using purified IF3N from Escherichia coli as a target. Pull-down and Microscale Thermophoresis (MST) assays were used to identify Apt^{343} as an aptamer candidate that specifically binds to IF3N with a K_d = 205 ± 164 nM. Rapid kinetics coupled to intramolecular Förster Resonance Energy Transfer (rkFRET) showed that Apt^{343} binds to a double-labeled IF3 drastically reducing by 13-fold the speed of IF3 binding to the 30S subunit. Additionally, the aptamer affected IF1- and IF2-depended conformational accommodations of IF3 on the 30S subunit. Furthermore, the canonical codon-anticodon duplex formation appeared to be delayed by Apt343. However, this delay does not affect the 50S joining reaction upon 70S IC formation. Altogether, our results suggest that IF3N positioning contributes to the accommodation of initiator tRNA and therefore, with canonical 30S IC formation. Additionally, the experimental scheme presented here provides a solid alternative for the development of new inhibitors of bacterial translation.