Increased versatility of protein synthesis through improved translation elongation factors

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Research objective

Cells use proteins with diverse functions and sequences. Because the amino acid sequences of proteins are encoded within DNA, the application of biological resources has traditionally followed a flow of: (1) identification of genes encoding useful proteins, (2) construction of genetically engineered organisms, and (3) mass production of target proteins. This process has been accelerated by the accumulation of genomic data through next-generation sequencing and, more recently, by high-precision prediction of protein structures using machine learning. However, although the discovery and development of useful proteins is rapidly advancing, little attention has been paid to whether such proteins can be synthesized within cells.

The ribosome, the cellular protein factory, has inherent "weaknesses" and is not capable of synthesizing all possible proteins. Nascent polypeptide chains traverse the peptide exit tunnel during synthesis, but some amino acid sequences can induce abnormal "distortions" within the ribosome owing to excessive interactions or repulsions [1, 2]. Examples of such "hard-to-translate" sequences include motifs with consecutive charged residues and stretches of prolines. To counteract these risks, cells have evolved and utilized translation-promoting factors to assist ribosomes that are perturbed by such sequences. In this study, I focused on *Escherichia coli* YbiT, a translation-promoting factor that facilitates the synthesis of hard-to-translate sequences enriched in positively or negatively charged amino acids. My goal was to engineer YbiT with enhanced translation-promoting activities beyond the natural form.

Methods

YbiT belongs to the ATP-binding cassette subfamily F (ABCF) protein family, of which four members exist in *E. coli*. Despite their highly similar three-dimensional structures, each member specifically acts on distinct, hard-to-translate sequences (Fig. 1) [3]

Functional defects induced by problematic amino acid sequences are thought to arise from structural abnormalities around the peptidyl transfer center of the ribosome. ABCF proteins are believed to resolve such defects by inserting their α-helix-rich linker domains into the ribosomal interior [4]. Therefore, YbiT is presumed to act through its linker domain to eliminate or prevent translational abnormalities. However, the precise underlying molecular

mechanisms remain unclear. In this study, I conducted a comprehensive mutational analysis (alanine scanning) of the linker domain to identify residues essential for YbiT function and obtain variants with enhanced translation-promoting activity.

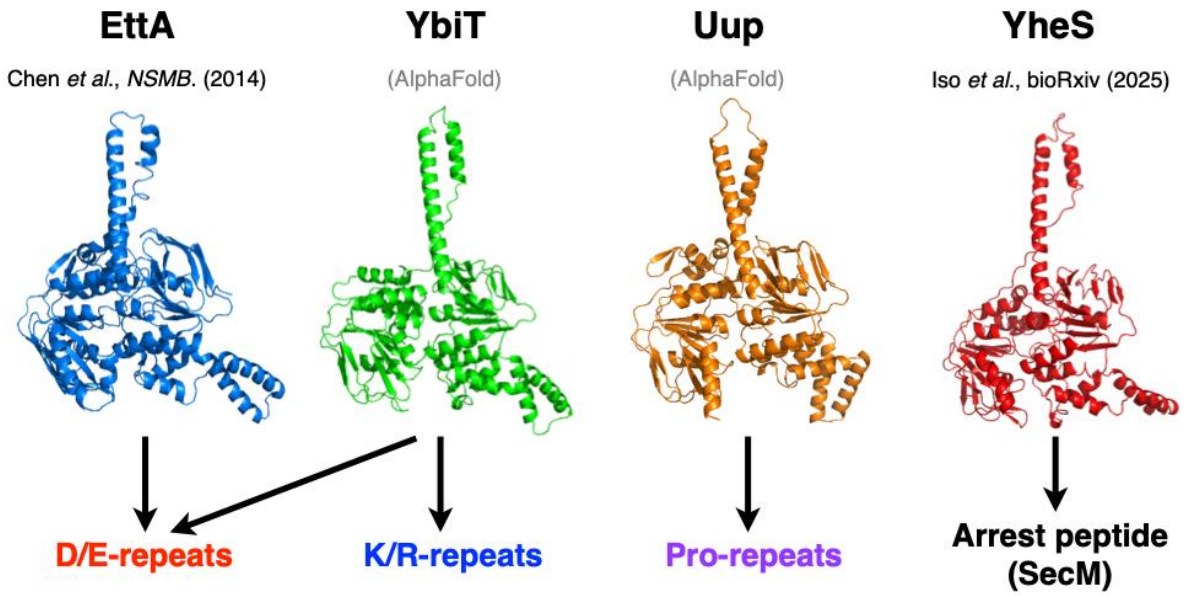

Fig. 1 Each ABCF protein in *E. coli* copes with distinct hard-to-translate sequences.

Results

Alanine substitution mutants were generated for all 85 residues of the YbiT linker domain, and their activities were evaluated using reporter genes that encode representative hard-to-translate sequences (Fig.2). Three distinct classes of problematic sequences were used, and the activity of each YbiT mutant was assessed.

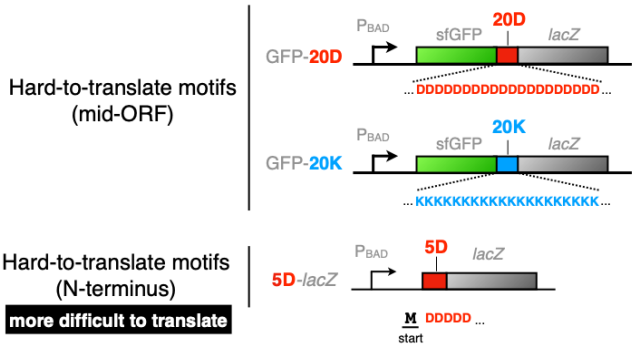

Fig. 2 Reporters used to evaluate the activity of YbiT mutants.

First, the ability of the mutants to promote the synthesis of sequences containing long stretches of negatively (20 D) or positively (20 K) charged residues within the ORF was compared. A strong correlation was observed (rho = 0.595, p = 1.82e^{-9}), indicating that the same residues in YbiT promoted the translation of both negatively and positively charged clusters.

Next, the translation promotion of a negatively charged cluster located immediately after the start codon (5D), which exacerbates ribosomal defects [5], was compared with that of the

internal 20D sequence. A similarly high correlation was observed (*rho* = 0.648, $p = 1.85e^{-10}$), suggesting that YbiT promotes the synthesis of negatively charged sequences via the same mechanism, regardless of whether the nascent peptide is present in the exit tunnel.

Conclusion

This study provides an overview of the amino acid residues in the YbiT linker domain that are critical for its function. Notably, mutants with severely reduced activity were concentrated at the tip of the linker, highlighting the importance of interactions within the ribosome. In addition, several variants exhibited higher translation-promoting activity than the wild-type. However, these enhanced mutants tended to act selectively on certain hard-to-translate sequences, and the underlying mechanism remains to be elucidated. Although only alanine substitutions were tested in this study, further large-scale mutational analyses are necessary to isolate YbiT variants with substantially improved activity.

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References

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