ABC-F Proteins Are Translation Factors That Rescue Context-Dependent Ribosomal Stalling from Early Elongation to Translation Termination

A. Wawszczyk[I], S. Ngo[I], D. Goicochea Paredes[I], L. Monlezun[I], **G. Boël**[I]

[I]EGM, CNRS, Université Paris Cité, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005, Paris, France

The cytosolic ATP Binding Cassette F (ABC-F) family, has been shown to orchestrate protein synthesis by mediating ribosomal activity in both eukaryote and prokaryote. One of the best characterised ABC-Fs is the energy sensing translational throttle A (EttA), one of four ABC-F proteins present in *Escherichia coli* (EttA, Uup, YbiT and YheS). EttA was shown to modulate the positioning of the ribosomal P-site tRNA and regulate peptidyl bond formation (Boël et al., 2014 *NSMB*, Singh *et al*., 2023, *bioRxiv*). Recent studies conducted in our laboratory demonstrated a role of EttA in regulation of expression of enzymes of the Krebs cycle's glyoxylate shunt pathway. Furthermore, identification of specific EttA targets revealed a peptide signature – the presence of negatively charged residues in the early coding sequence (Ousalem et *al*., 2024, *Nat. commun*).

Mechanisms behind the other *E. coli* ABC-Fs are more elusive and each of the factors is proposed to have unique function in the cell. For the Uup, the involvement in DNA recombination and replication has been previously reported (Reddy *et al*., 2000, *J Bacteriol*; Romero *et al*., 2020, *NAR*). While the direct interaction of Uup with DNA has been debated, structural analysis demonstrated binding of the Uup to the ribosomal E-site (Singh *et al*., 2023, *bioRxiv*).

In order to decipher the nature of the Uup-mediated regulation on the DNA replication and recombination we employed a selection of cross-sectional *in vivo* and *in vitro* methodologies including global-omics approaches that the results of have been biochemically validated. The findings of the study demonstrated that production of some of the indispensable DNA replication factors is under a Uup-driven translational control. This regulation occurs during translation termination and is selective to sequences that contain amino acids that has previously been demonstrated to induce ribosomal stalling. Moreover, Uup was found to prevent the action of the tmRNA ribosomal rescue system on mRNA under Uup-driven translational control. Our results show that Uup rescues problematic translation termination and demonstrate that its involvement in DNA replication and recombination is indirect, acting through the translational regulation of DNA replication factors.