The Ribosome – Ligand Interactions and Dynamics as Inferred by Cryo-EM

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Protein synthesis involves the processive interaction of multiple ligands (mRNA, tRNA, EF-Tu, EF-G) with the ribosome, coupled with conformational changes of both ribosome and ligand molecules. Cryo-electron microscopy combined with three-dimensional reconstruction of single particles is ideally suited to study these processes during the main phases of translation: initiation, elongation, and termination. Thus far, the elongation cycle, in the course of which a new amino acid is added to the nascent polypeptide chain, has received the most attention. It is driven by the alternate binding of (i) the ternary complex consisting of elongation factor Tu (EF-Tu), aminoacyl-tRNA, and GTP, and (ii) elongation factor G (EF-G). The first of these binding interactions catalyzes the incorporation of a new, cognate tRNA into the A site, while the second catalyzes tRNA translocation (A->P, P->E, E->out). Both interactions require GTP hydrolysis.

Cryo-EM has been used to investigate both interactions, taking advantage of antibiotics that prevent the release of the factors. As expected from the close similarity of the two ligands ("molecular mimicry" [1]), they bind at the same location on the ribosome [2-5], but this immediately poses a problem in explaining the alternate binding modes. However, closer analysis using new cryo-EM data on the binding of ternary complex [6] and EF-G in the GTP form [7] shows that the residues contacting the ribosome form different constellations for the two factors. This coincides with the observation that EF-G binding (in the presence of a noncleavable GTP analog) triggers a large conformational change, described as a rotational ratchet motion between the two ribosomal subunits [8]. Tentatively, then, the elongation cycle might involve a sequence of the following kind [9]: factor A binds to the ribosome, performs its work, triggering a conformational change that destabilizes its own binding and leads to a conformation that invites factor B; factor B binds to the ribosome, etc. This concept suggests further experiments, but requires that cryo-EM advances toward higher resolution.

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[10] Supported by Howard Hughes Medical Institute, NIH R37 GM29169 (to JF) and R01 GM61576 (to RKA)

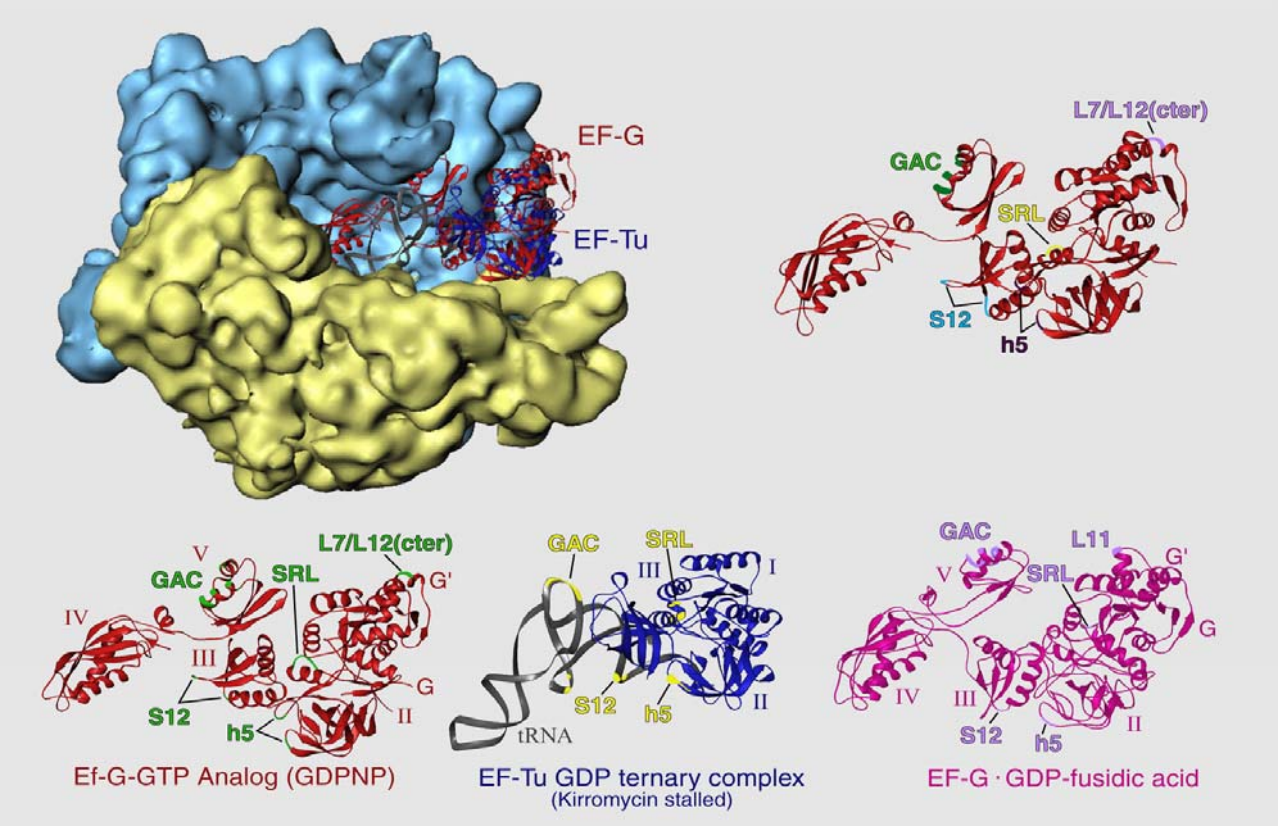

Fig. 1. Top: 70S ribosome from *E. coli*, with overlaid X-ray structures of EF-G (GDP form, with fusidic acid) and aa-tRNA·EF-Tu·GDP·kirromycin. Bottom: comparison of ternary complex with EF-G in both GDP and GTP forms. The comparison shows that the residues known to contact the ribosome at equivalent sites (indicated by labels) form different constellations for all three structures, implying that the ribosome *must* change its conformation to accommodate the binding of the factors in succession.

https://doi.org/10.1017/S1431927602100031 Published online by Cambridge University Press