Structured Abstract

Introduction

During protein synthesis, the GTPase elongation factor G (EF-G) promotes translocation of messenger RNA (mRNA) and transfer (tRNA) on the ribosome. Translocation requires multiple steps that involve large-scale rearrangements of the ribosome that are directed by EF-G. The structural basis for how the GTPase active site in EF-G, composed of mobile "switch" elements that coordinate the GTP triphosphate and positioned by the large (50S) ribosomal subunit, connects to events on the small (30S) subunit remain unclear at a molecular level.

Methods

Ribosomes from *Escherichia coli* lacking the C-terminal region of protein L9 were complexed with EF-G, the nonhydrolyzable GTP analog GMPPCP and viomycin. Two crystal forms, each containing four unique copies of the ribosome/EF-G/GMPPCP complex, along with different stoichiometries of bound viomycin were used to solve eight ribosome structures by molecular replacement using diffraction data to 3 Å resolution. After structure refinement, the ribosome complexes were superimposed with each other and with prior x-ray crystal structures and cryo-electron microscopy reconstructions of the ribosome to identify the effects of EF-G/GMPPCP binding.

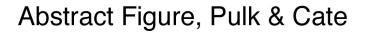
Results

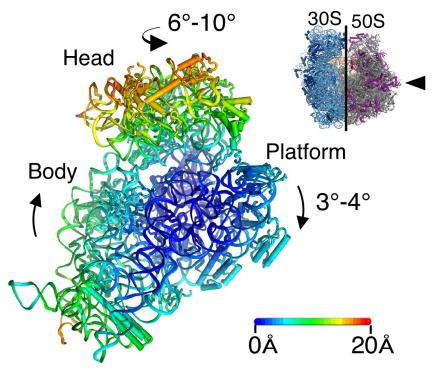
Binding of EF-G in the GMPPCP state to the ribosome orders switch elements in the GTPase active site that are unfolded in the GDP state. The GTPase switch elements contact the 50S subunit in an activated conformation, as seen in ribosome structures with elongation factor Tu (EF-Tu) and aminoacyl-tRNA during mRNA decoding. Folding of the switch regions also causes EF-G to adopt a rigid conformation with numerous interdomain contacts. In the structures, EF-G/GMPPCP binds the ribosome in three different states of ribosomal subunit rotation, but is more ordered in the intermediate and fully rotated states than in the unrotated state. Domain IV of EF-G is positioned in the aminoacyl-tRNA binding site (A site) on the small ribosomal subunit, where mRNA decoding occurs, but does not contact the head domain of the 30S subunit.

Discussion

In the present ribosome structures, EF-G/GMPPCP adopts a rigid conformation that positions EF-G domain IV in the 30S subunit A site, a conformation incompatible with early steps of translocation. The observed rigid conformation of EF-G may represent the activated GDP•P_i form, with EF-G domain IV decoupling tRNA movement from the 30S subunit platform and allowing the intrinsic dynamics of the 30S subunit head domain to translocate tRNAs into the P and E sites. After GTP hydrolysis and inorganic phosphate (P_i) dissociation, unfolding of the GTPase center in EF-G would release inter-domain contacts in a trajectory that matches that of aminoacyl-tRNA release from EF-Tu during mRNA decoding. The relaxed state of EF-G/GDP would then allow the 30S subunit to revert to the unrotated conformation, and EF-G/GDP to dissociate from the ribosome.

Structured Abstract Figure. (Left) Ribosome in an intermediate state of 30S subunit rotation stabilized by EF-G bound to GMPPCP, viewed from the 50S subunit (inset). Color-coding is by the distance between corresponding atoms in the unrotated state of the ribosome. (**Right**) Opening of domains II and III in EF-G after GTP hydrolysis follows the trajectory of tRNA release from EF-Tu during mRNA decoding.





Intermediate rotation

