The translocation of the peptidyl- and deacetylated tRNAs from the A and P to P and E ribosomal sites is catalyzed by the translation elongation factor G (EF-G). EF-G represents a GTPase, which hydrolyzes GTP at a certain step of its function on the ribosome. How the GTPase activity of EF-G is regulated remains unknown. Based on systematic comparison of the available X-ray structures of ribosome complexes with EF-G, we demonstrate that the activation of the EF-G-dependent GTPase represents a multi-step process, which includes such an unexpected step as a deep insertion of a nucleotide of ribosomal RNA into the body of the factor.