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Supplemental Data

Reverse Translocation of tRNA in the Ribosome

Shinichiro Shoji, Sarah E. Walker, and Kurt Fredrick

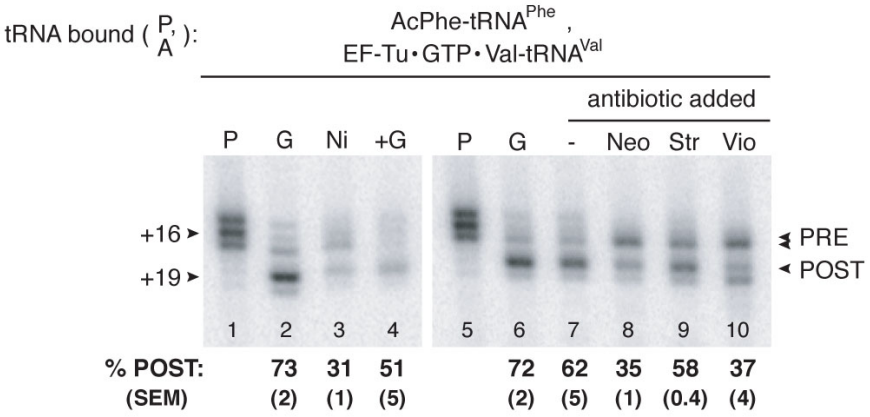

Figure S1. Conversion of ribosomal complexes containing $tRNA^{Phe}$ and *N*-acetyl-Phe-Val-$tRNA^{Val}$ from the POST to PRE state by depletion of EF-G-His6 or by addition of antibiotics. The POST complex was made in polymix buffer by binding *N*-acetyl-Phe-$tRNA^{Phe}$ to the P site of m617-programmed ribosomes (P lanes) and then adding a mixture of ternary complex (EF-Tu•GTP•Val-$tRNA^{Val}$), EF-G-His6, and GTP (G lanes). EF-G-His6 was then depleted from the reaction using Ni^{2+}-agarose (Ni lane). Alternatively, antibiotics were added to the reaction as indicated. Evidence that the +16/17 complex corresponded to the PRE complex was provided by re-addition of EF-G and GTP after Ni^{2+}-agarose treatment (+G lane). A multiple-step translocation assay [Takyar, S., Hickerson, R.P., and Noller, H.F. (2005). mRNA helicase activity of the ribosome. Cell *120*, 49-58.] confirmed that A-site binding was EF-Tu-dependent under these conditions.

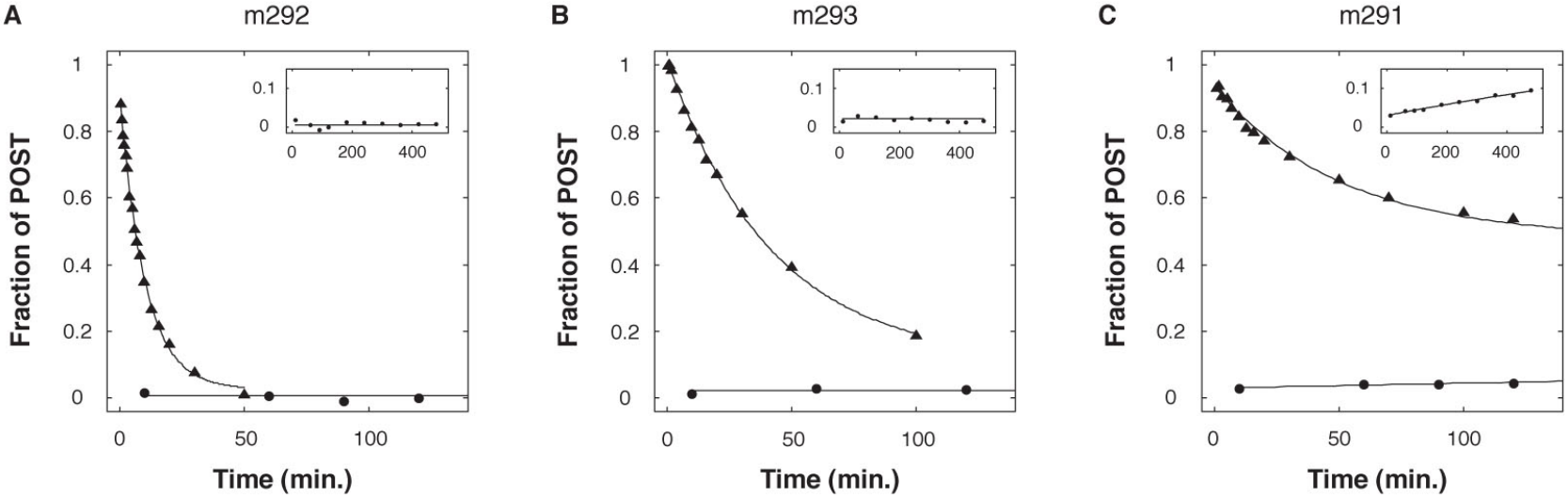

Figure S2. Time courses to monitor spontaneous PRE to POST (circles) and POST to PRE (triangles) conversion under otherwise identical conditions in the m292 (panel A), m293 (panel B) or m291 (panel C) context. To measure PRE to POST conversion, programmed ribosomes (0.7 μM) were incubated with deacylated $tRNA^{fMet}$ (1 μM) to bind the P site, and *N*-acetyl-aminoacyl-tRNA cognate for the A codon (1 μM) was subsequently added at $t = 0$. To measure POST to PRE conversion, programmed ribosomes (0.7 μM) were incubated with *N*-acetyl-aminoacyl-tRNA (1 μM) to bind the P site, and deacylated $tRNA^{fMet}$ (1 μM) cognate for the E codon was subsequently added at $t = 0$. Complexes were formed in TNM buffer to promote high occupancy of the A and E sites. Insets: Extended time courses to monitor PRE to POST conversion.