Supporting Information

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SI Materials and Methods

Chemicals were purchased from Sigma, Roche, or Merck, and radioactive compounds were from Hartmann Analytic. mant-GTP was from Jena Bioscience. MFV-mRNA (5′-GGCAAG-GAGGUAAAUAAUGUUCGUUAUU-3′; the coding sequence is underlined) was purchased from IBA. The mutations H84Q, H84R, D21A, D21N, and D21E were inserted by site-directed mutagenesis of the pET24a plasmid containing the *Escherichia coli tufA* gene. The H84A mutant was reported earlier (1). C-terminally His-tagged elongation factor Tu (EF-Tu) and EF-Tu mutants were expressed in *E. coli* BL21 (DE3) cells and purified as previously described (1). Ribosomes, [^{14}C]Phe-tRNA^{Phe}, and f[^{3}H]Met-tRNA^{fMet} were prepared as described (2). Initiation complexes (70S) were prepared by incubating ribosomes (1 μM) with f[^{3}H]Met-tRNA^{fMet} (2 μM) and mRNA (3 μM) in the presence of initiation factors IF1, IF2, and IF3 (1.5 μM each) for 30 min at 37 °C. Initiation complexes were purified by centrifugation through 1.1 M sucrose cushions in buffer A (50 mM Tris·HCl, pH 7.5, 70 mM NH_4Cl, 30 mM KCl, 7 mM $MgCl_2$) at 259,000 × *g* for 2 h in a Beckmann Optima Max-XP ultracentrifuge. Binding of f[^{3}H]Met-tRNA^{fMet} to the P site was quantified by nitrocellulose filtration. Ternary complex EF-Tu–GTP–Phe-tRNA^{Phe} was prepared and purified as described (3). For kinetic solvent isotope effect experiments, a buffer-exchange step using Nap5 Sephadex columns (GE Healthcare) was included after gel filtration. Quench-flow assays were performed by rapidly mixing equal volumes (14 μL) of purified ternary complex (0.2 μM) and initiation complex at the indicated concentrations using a KinTek quench-flow apparatus. Stopped-flow experiments were performed in an SX-20MV apparatus (Applied Photophysics). The fluorescence of proflavin (Prf) and Bodipy-FL (BOF-nucleotides) was excited at 470 nm and that of mant-GTP was excited at 363 nm, and data were collected after passing through a KV500 filter (Schott) for Prf and BOF-nucleotides or KV408 for mant-GTP. Time courses were obtained by averaging four to eight individual traces. Purified ternary complexes EF-Tu–mant-GTP–Phe-tRNA^{Phe} or EF-Tu–GTP–Phe-tRNA^{Phe}(Prf) (0.2 μM) were mixed with initiation complexes at the indicated concentrations. The K^+ ion dependence of the intrinsic GTPase activity was determined. The Hill plot in Fig. 3*D* was generated by plotting the difference in the rates of GTP hydrolysis with WT and D21A EF-Tu at each KCl concentration (up to 1 M) against the KCl concentration.

1. Daviter T, Wieden H-J, Rodnina MV (2003) Essential role of histidine 84 in elongation factor Tu for the chemical step of GTP hydrolysis on the ribosome. *J Mol Biol* 332(3): 689–699.
2. Rodnina MV, Wintermeyer W (1995) GTP consumption of elongation factor Tu during translation of heteropolymeric mRNAs. *Proc Natl Acad Sci USA* 92(6):1945–1949.
3. Rodnina MV, Fricke R, Wintermeyer W (1994) Transient conformational states of aminoacyl-tRNA during ribosome binding catalyzed by elongation factor Tu. *Biochemistry* 33(40):12267–12275.

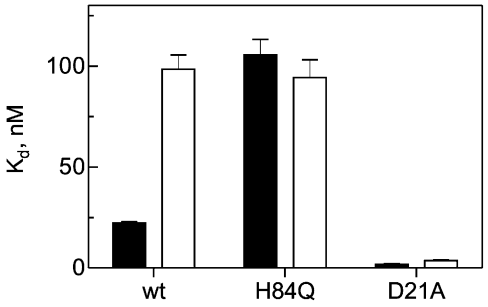

Fig. S1. Nucleotide-binding affinity of WT, H84Q, and D21A EF-Tu. EF-Tu–bound GDP was first removed by incubation for 30 min at 37 °C with 50 mM EDTA, and the nucleotide-free proteins were purified on Nap5 Sephadex columns (GE Healthcare). The kinetics of nucleotide binding was monitored upon mixing of nucleotide-free EF-Tu (0.25 μM) and BOF-GDP or BOF-GTP (0.31–5 μM). Association rate constants, k_{on}, were determined from the concentration dependence of the apparent rate constants. Dissociation rate constants, k_{off}, were determined by chase, upon mixing EF-Tu, preincubated with BOF-nucleotides, and purified through Nap5 columns, with a large excess (25 μM) of the respective unlabeled nucleotide. K_d values for GDP (black bars) and GTP (white bars) were determined from the k_{off}:k_{on} ratio. Error bars indicate the SD of linear fitting.

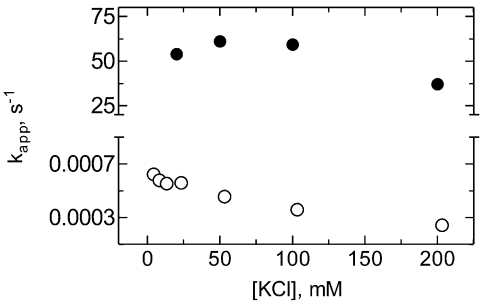

Fig. S2. K^+ ion dependence of WT EF-Tu GTPase activity in the presence of ribosomes. Apparent rate constants (k_{app}) of GTP hydrolysis in the presence of programmed ribosomes (closed circles) were determined upon mixing of purified ternary complexes (0.2 μM) with initiation complexes (0.6 μM) at the indicated concentration of KCl. GTPase activity in the presence of nonprogrammed ribosomes (1.2 μM) (open circles) was determined as in Fig. 3*C* using free EF-Tu (0.4 μM).

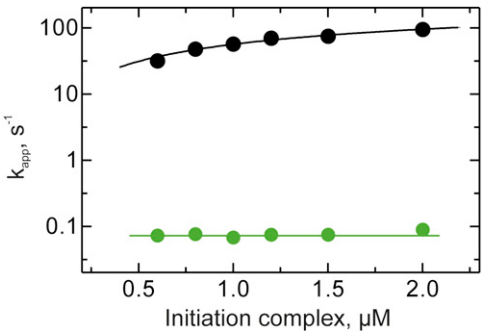

Fig. S3. Concentration dependence of GTP hydrolysis of WT and D21A EF-Tu. Kinetics of GTP hydrolysis was measured with WT (black circles) and D21A (green circles) purified ternary complex (0.2 μM) and increasing concentrations of programmed ribosomes.