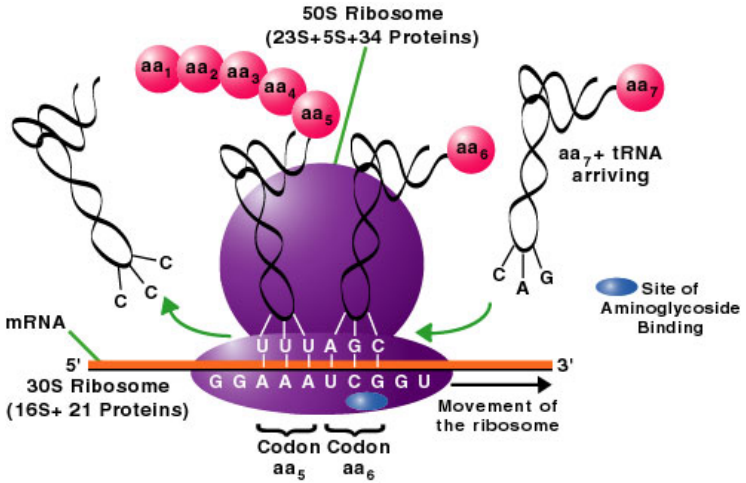

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Spontaneous Intersubunit Rotation in Single Ribosomes

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SUMMARY

During the elongation cycle, tRNA and mRNA undergo coupled translocation through the ribosome catalyzed by elongation factor G (EF-G). Cryo-EM reconstructions of certain EF-G-containing complexes led to the proposal that the mechanism of translocation involves rotational movement between the two ribosomal subunits. Here, using single-molecule FRET, we observe that pretranslocation ribosomes undergo spontaneous intersubunit rotational movement in the absence of EF-G, fluctuating between two conformations corresponding to the classical and hybrid states of the translocational cycle. In contrast, posttranslocation ribosomes are fixed predominantly in the classical, nonrotated state. Movement of the acceptor stem of deacylated tRNA into the 50S E site and EF-G binding to the ribosome both contribute to stabilization of the rotated, hybrid state. Furthermore, the acylation state of P site tRNA has a dramatic effect on the frequency of intersubunit rotation. Our results provide direct evidence that the intersubunit rotation that underlies ribosomal translocation is thermally driven.

INTRODUCTION

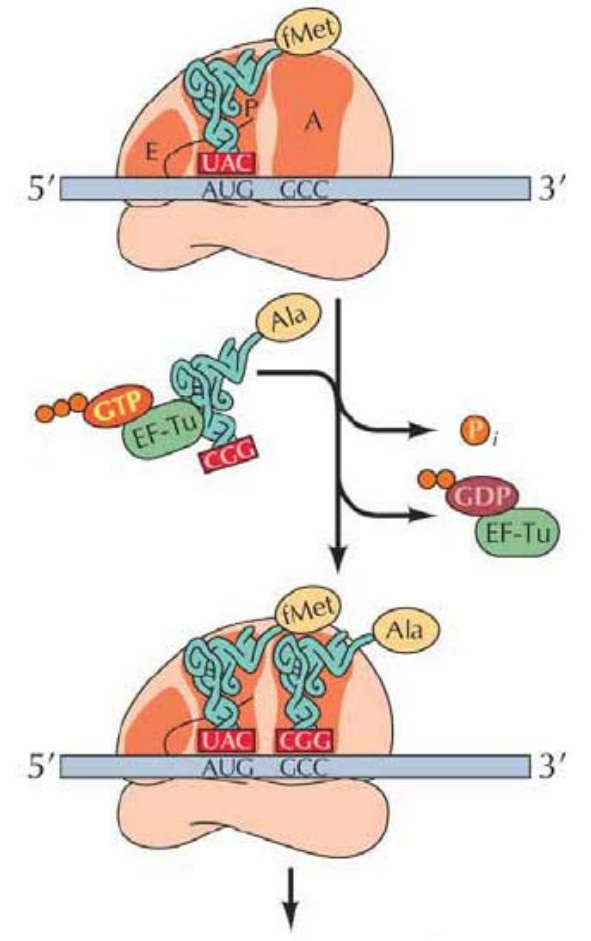
Protein synthesis is a dynamic process carried out by the ribosome, an RNA-based molecular machine. During protein synthesis, tRNA and mRNA are translocated through the ribosome in a series of complex, large-scale molecular movements catalyzed by elongation factor G (EF-G) and GTP. However, translocation can occur, albeit very slowly, in the absence of EF-G and GTP (Cukras et al., 2003; Fredrick and Noller, 2003; Gavrilova et al., 1976; Gavrilova and Spirin, 1971; Pestka, 1969). Thus, translocation is a property of the ribosome itself, rather than of EF-G, and is thermodynamically favored even in the absence of GTP hydrolysis.

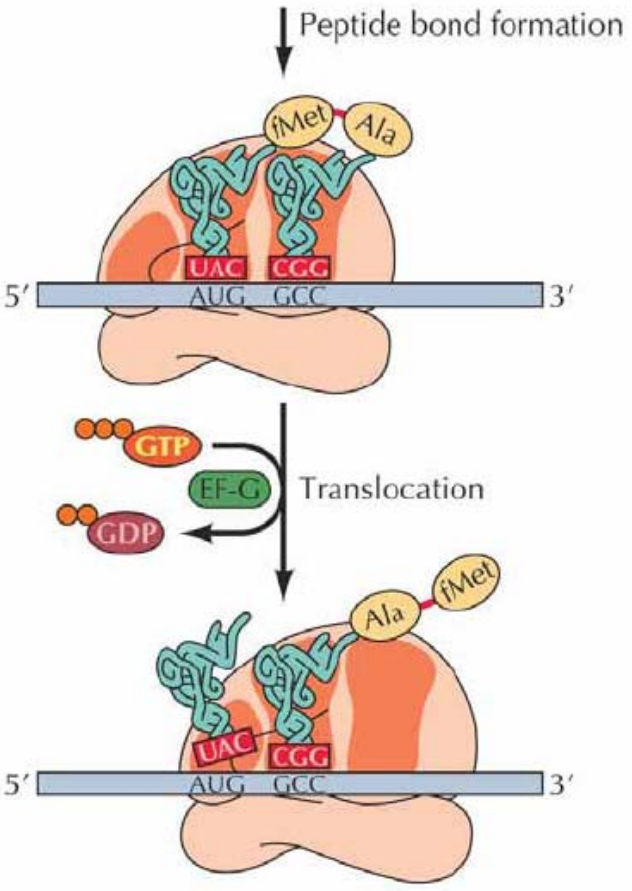
Chemical probing studies provided the first direct evidence that translocation takes place in two steps involving an intermediate hybrid state (Moazed and Noller, 1989b). In the first step, the acceptor ends of the tRNAs move relative to the 50S subunit, from their classical A/A- and P/P-binding states into hybrid A/P and P/E states (in which the peptidyl-tRNA is bound in the 30S A site and the 50S P site and the deacylated tRNA is bound in the 30S P site and the 50S E site; Figure 1A). The specific affinity of the acceptor end of deacylated tRNA for the 50S E site (Lill et al., 1986) helps to account for the thermodynamic driving force for spontaneous formation of the hybrid state. In the second step, which strongly depends on participation of EF-G, their anticodon ends move on the 30S subunit, coupled with mRNA movement, into the posttranslocational P/P and E/E states.

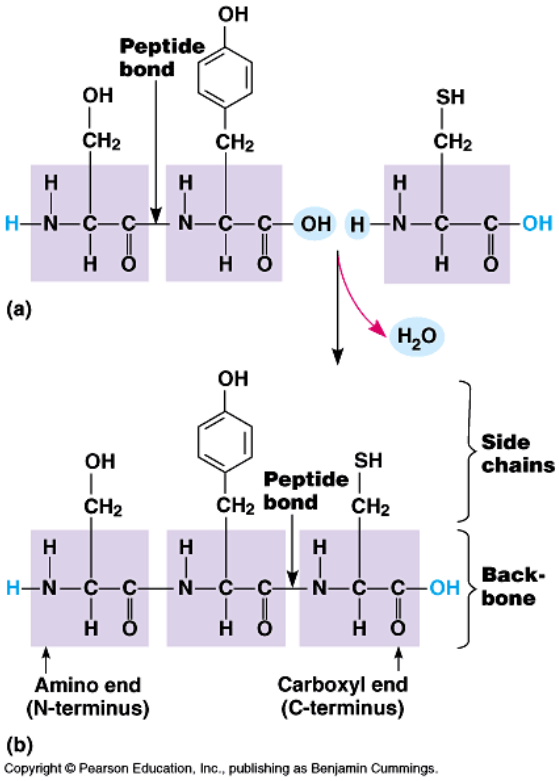
Cryo-EM studies have identified a conformation of the ribosome in which the 30S subunit is rotated by about 3°–10° counterclockwise relative to the 50S subunit in complexes containing EF-G·GDPNP (a nonhydrolyzable analog of GTP) or EF-G·GDP·fusidic acid (Frank and Agrawal, 2000; Gao et al., 2003; Valle et al., 2003). This finding led to the proposal of a ratchet-like mechanism, in which translocation of tRNA and mRNA is linked to intersubunit rotational movement (Frank and Agrawal, 2000; Frank et al., 2007; Tama et al., 2003; Valle et al., 2003). Recently, this model has been directly tested by formation of a disulfide bridge between ribosomal proteins S6 and L2 designed to restrict intersubunit movement, resulting in a specific block in translocation (Horan and Noller, 2007).

The hybrid-state and ratchet models have now converged. Recent bulk FRET measurements combined with chemical probing experiments show that the EF-G-induced rotation of the 30S subunit observed in cryo-EM reconstructions corresponds to formation of the hybrid state characterized by chemical probing studies (Ermolenko et al., 2007a, 2007b). Although EF-G binding was found to stabilize the rotated, hybrid state (Spiegel et al., 2007), rotation of the 30S subunit was also observed in the absence of EF-G under conditions favoring the hybrid state (Ermolenko et al., 2007a), consistent with previous biochemical experiments with pretranslocation complexes (Sharma et al., 2004). Furthermore, spontaneous movement of two fluorescently labeled tRNAs relative to each other, interpreted as movement of the tRNAs between the classical and hybrid states, was observed in individual pretranslocation ribosomes using single-molecule FRET (smFRET) (Blanchard et al., 2004b; Kim et al., 2007; Munro et al., 2007).

Translation

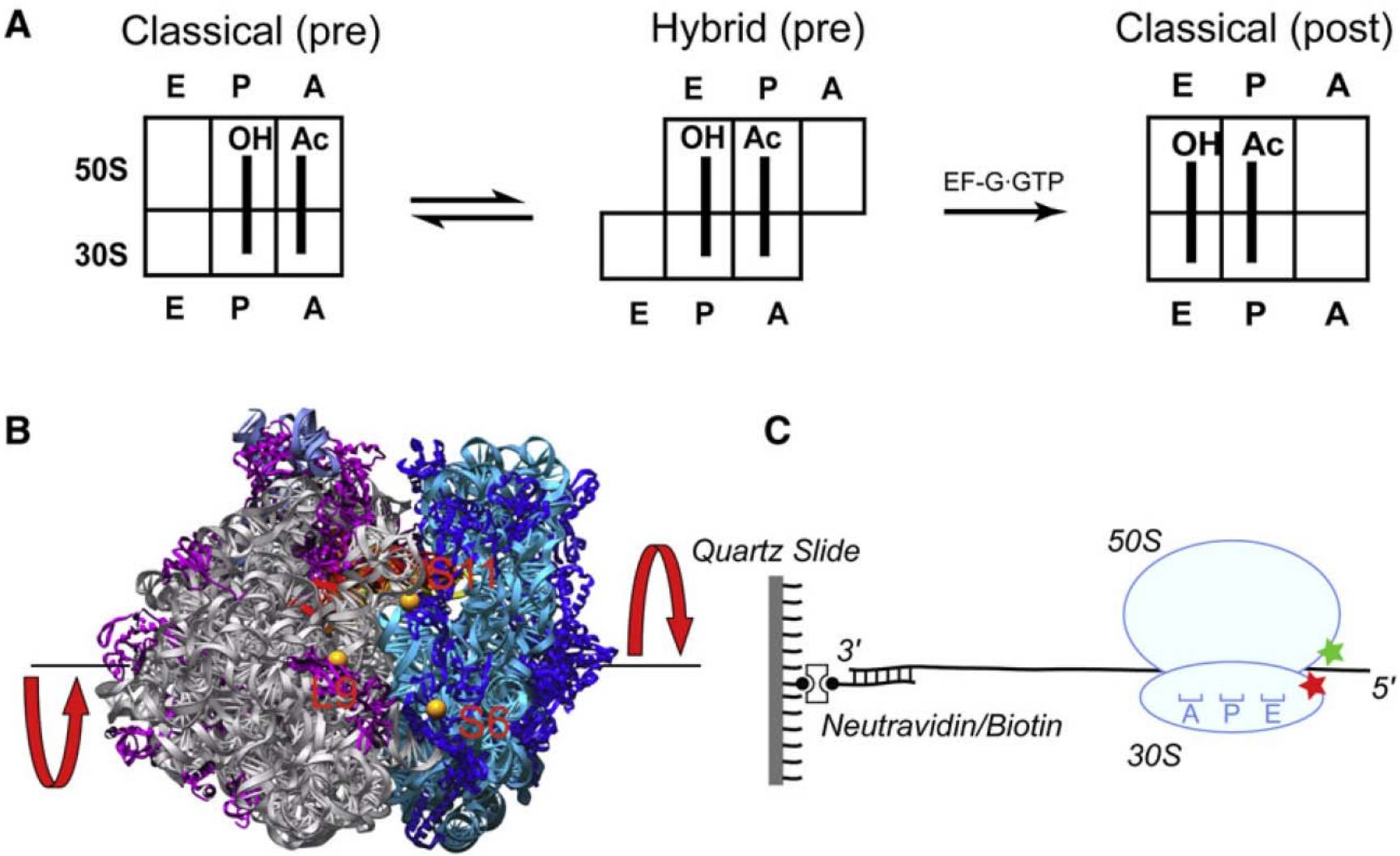




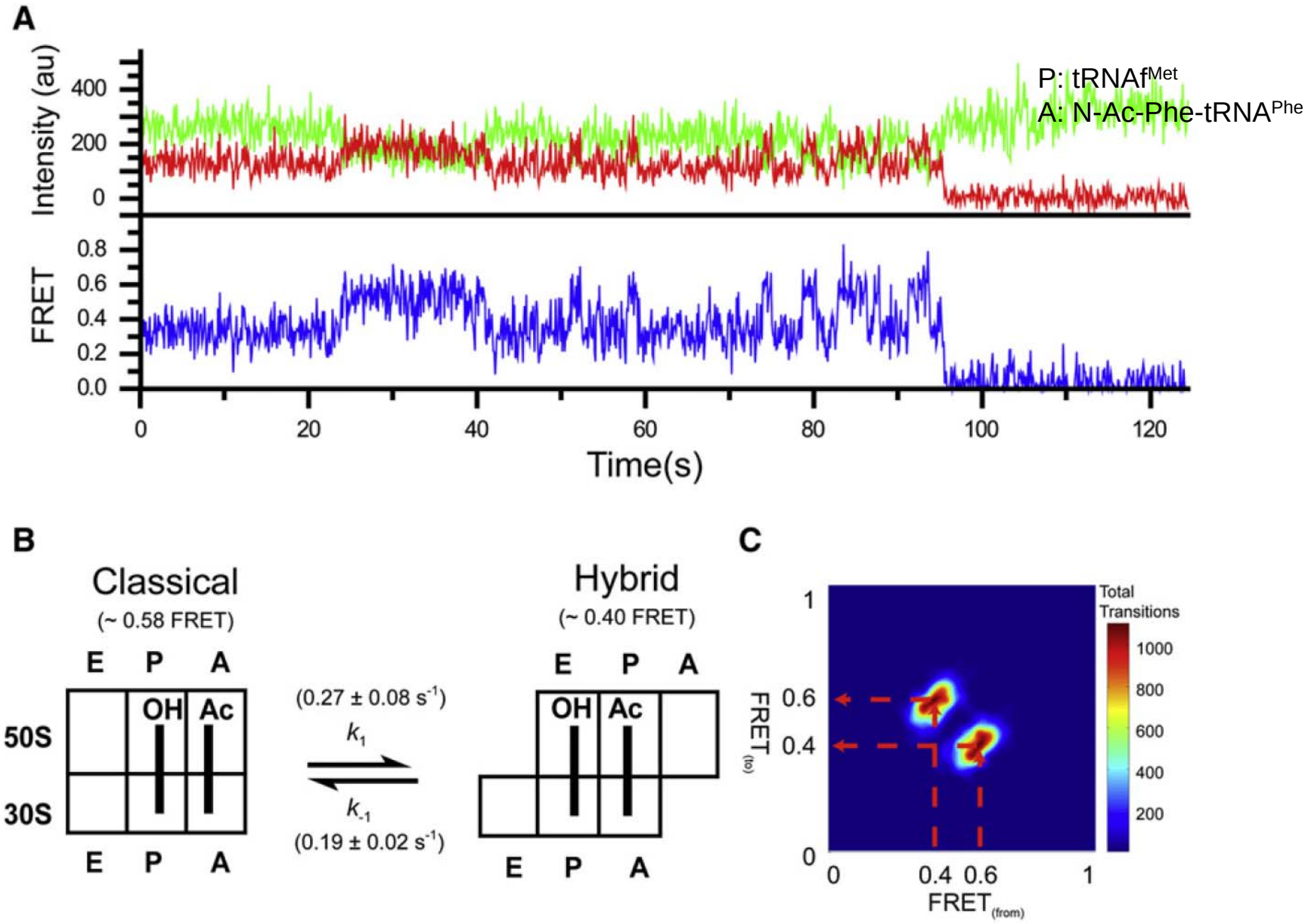


http://www.sumanasinc.com/webcontent/animations/content/translation.html

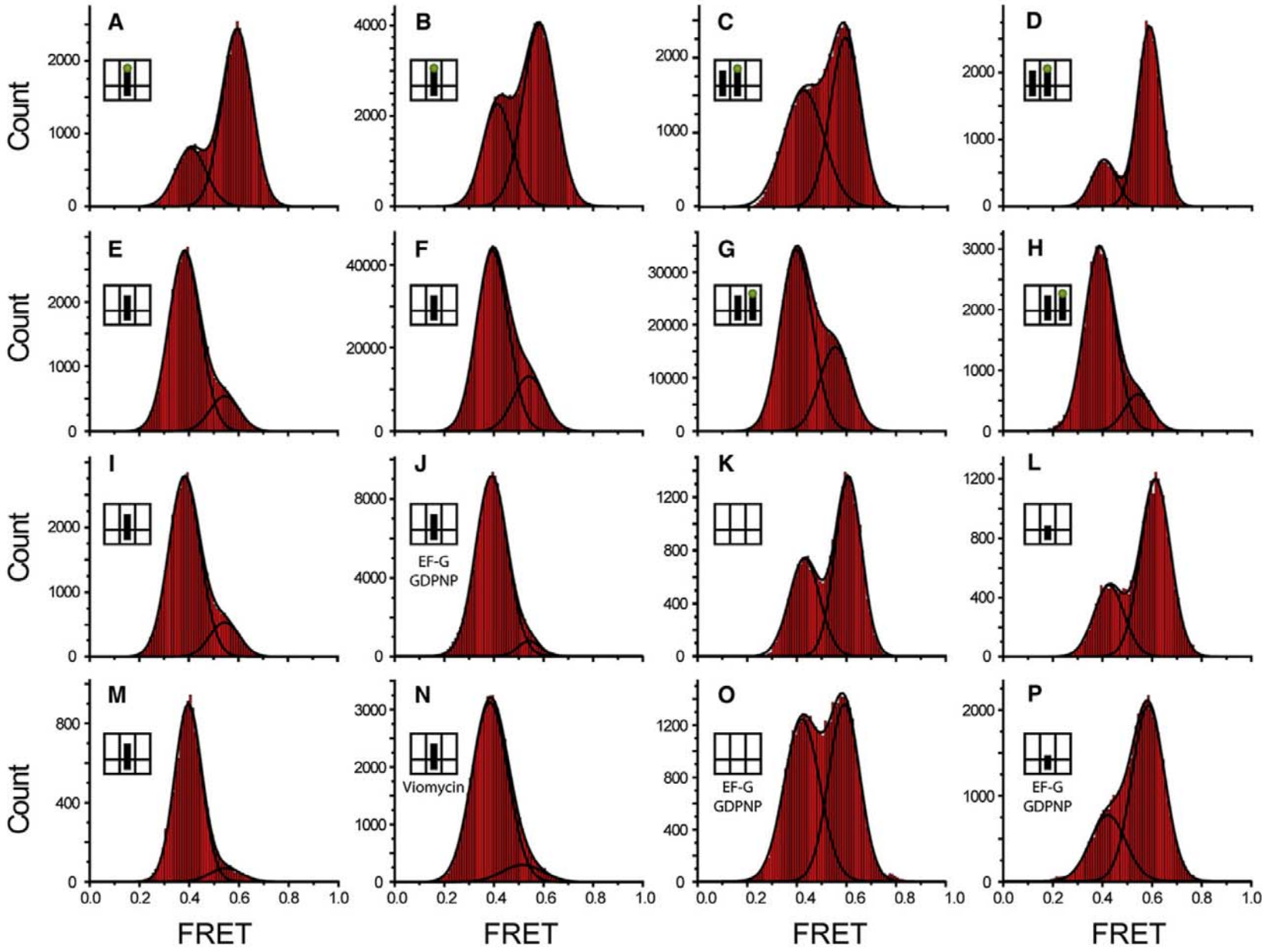
Subunit rotation of ribosome



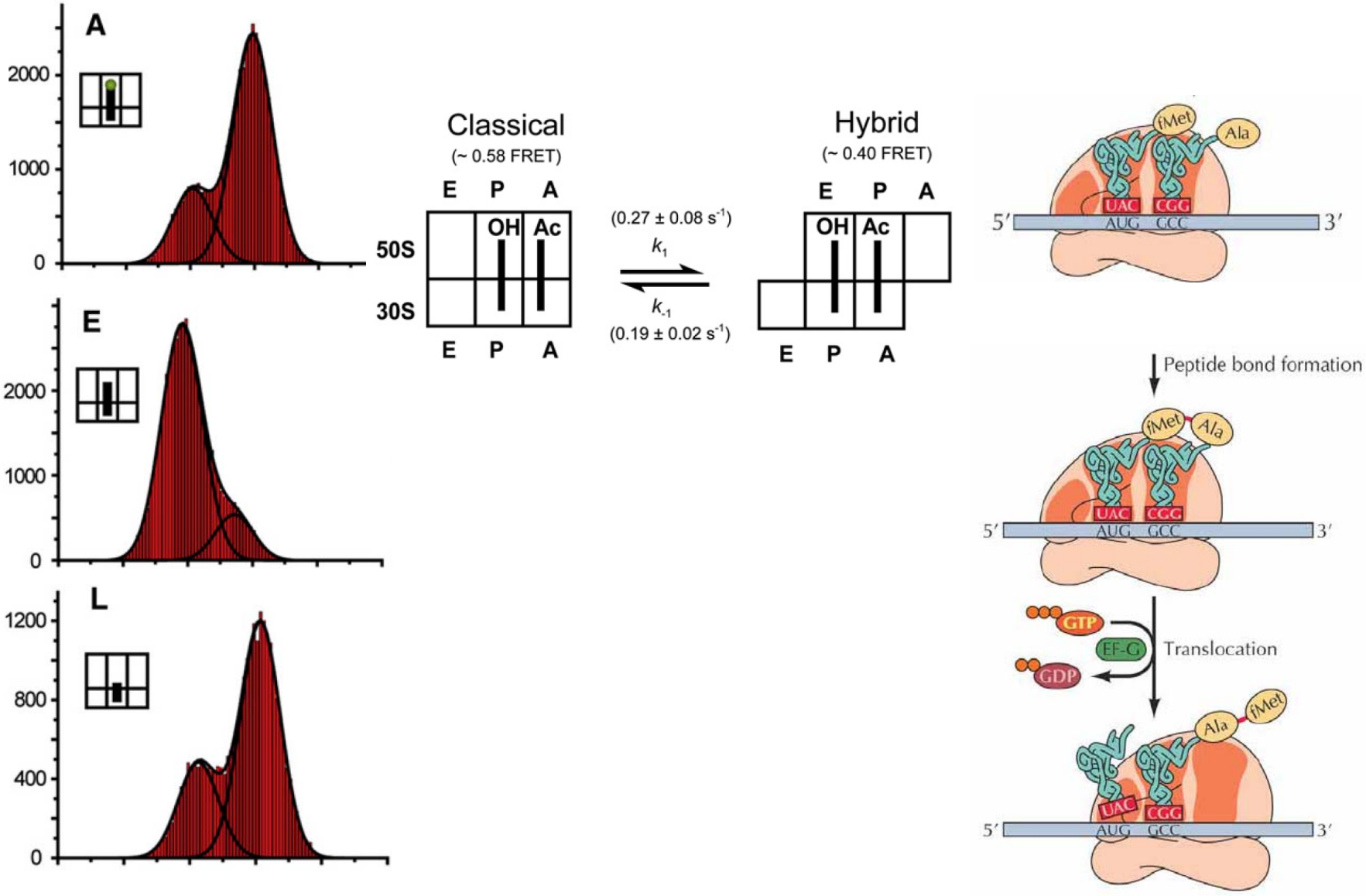
S6-Cy5/L9-Cy3 Pretranslocation ribosomes



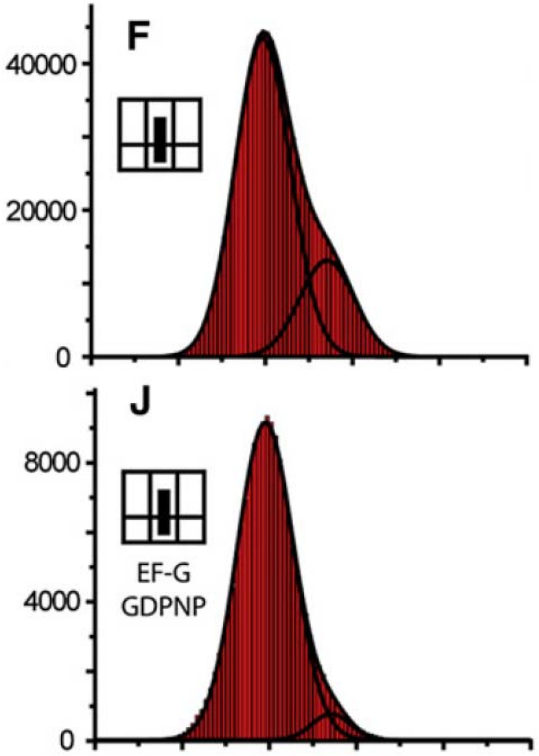
Effect of the Acylation state of the P site tRNA and EF-G

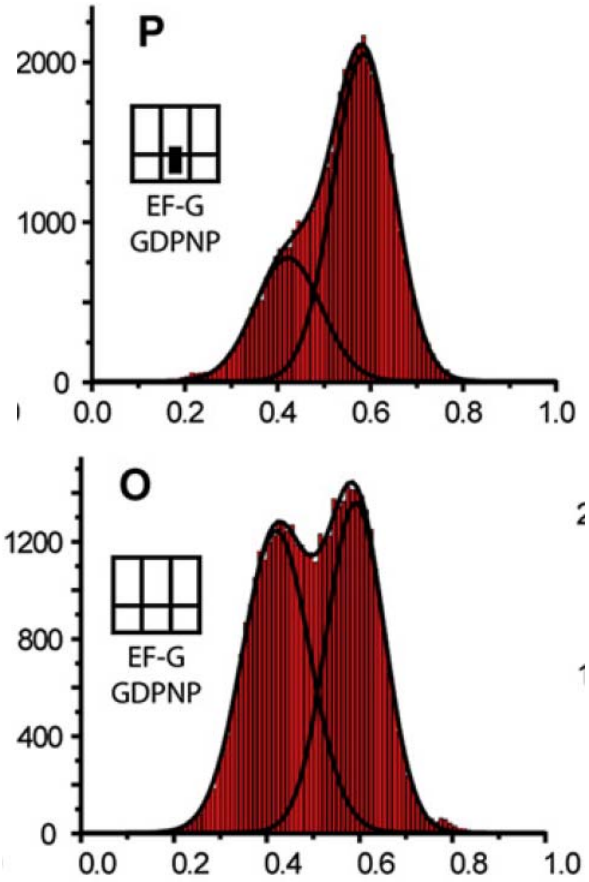


Interaction of the elbow or acceptor end of a deacylated tRNA with 50S E site promote stabilization of the P/E hybrid state

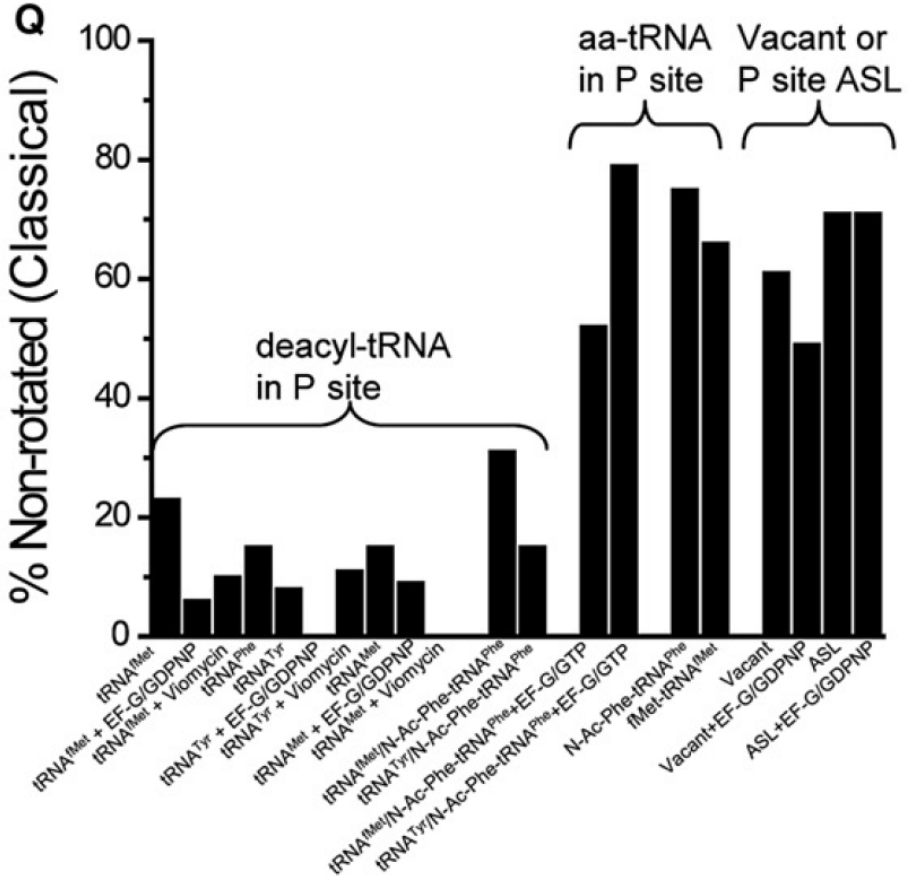


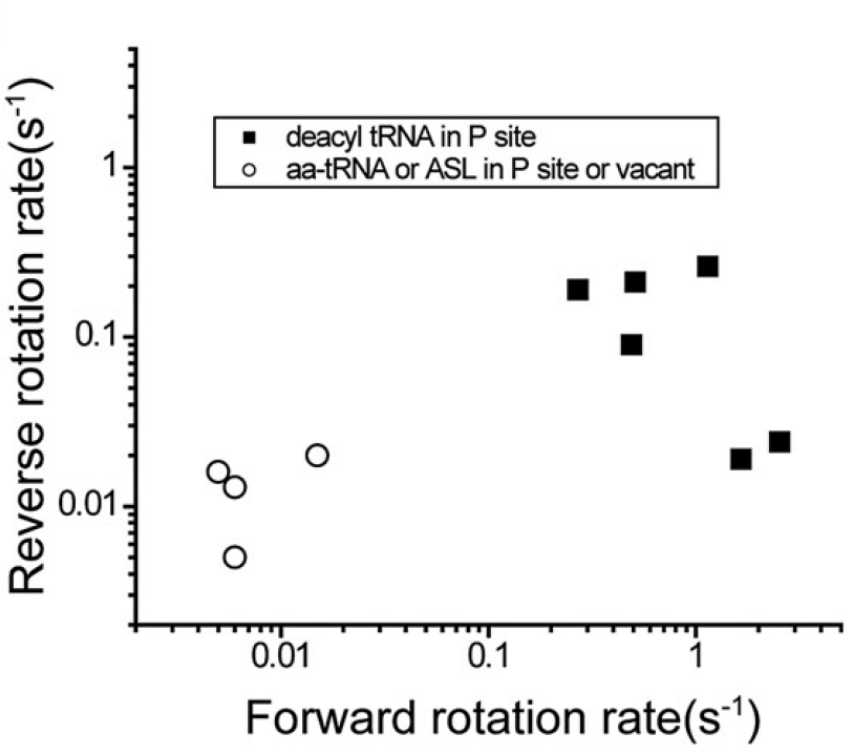
EF-G·GDPNP and viomycin coverted ribosomes into the rotated state





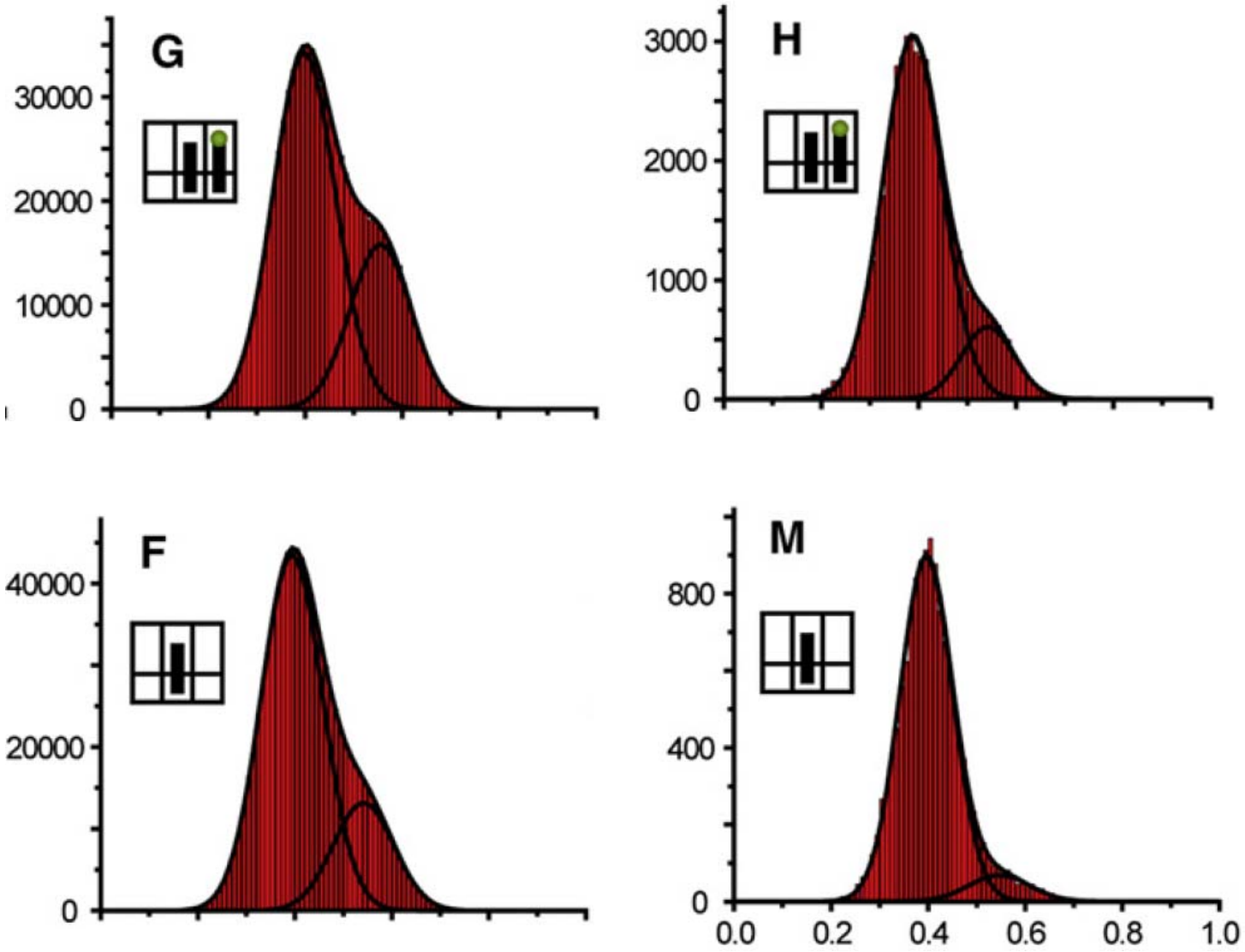
- Deacylated tRNA in the P site contributes to stabilization of the roteted hybrid state
- EF-G alone is insufficient to convert all ribosomes to the hybrid state



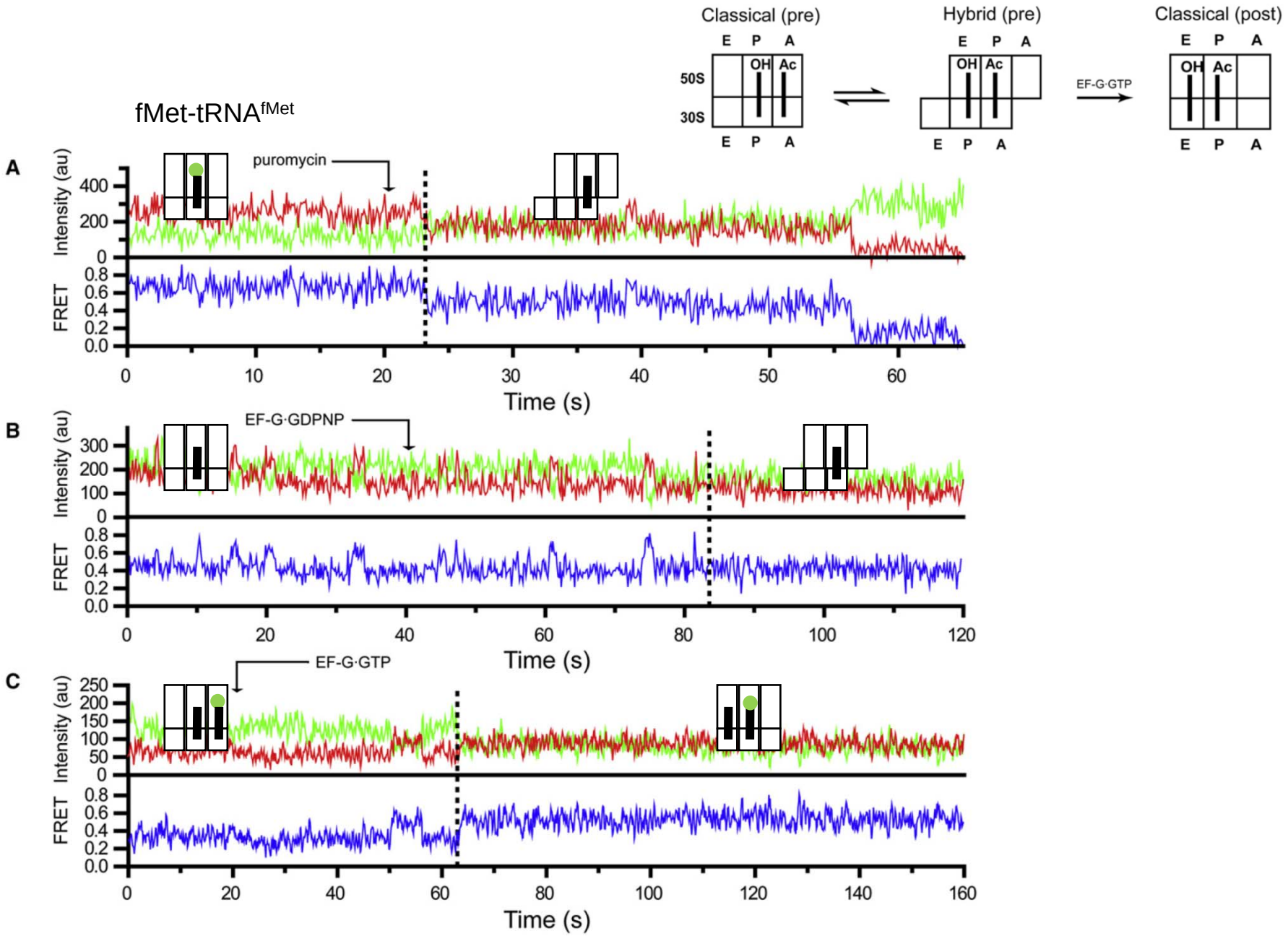


Presence of deacylated tRNA in P site changes kinetic rates

Addition of tRNA to the A site does not affect on kinetics much.
The identity of the Psite tRNA can influence kinetics.



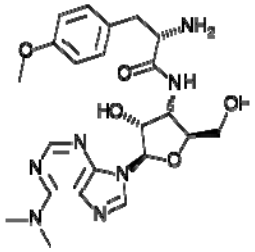
Real-time observation of subunit rotation



Conclusion

- The intersubunit rotational states are in dynamic equilibrium
- The P site tRNA significantly impacts ribosome dynamics
- pretranslocation ribosomes undergo
  spontaneous inter-subunit rotational movement w.o. EF-G

Puromycin



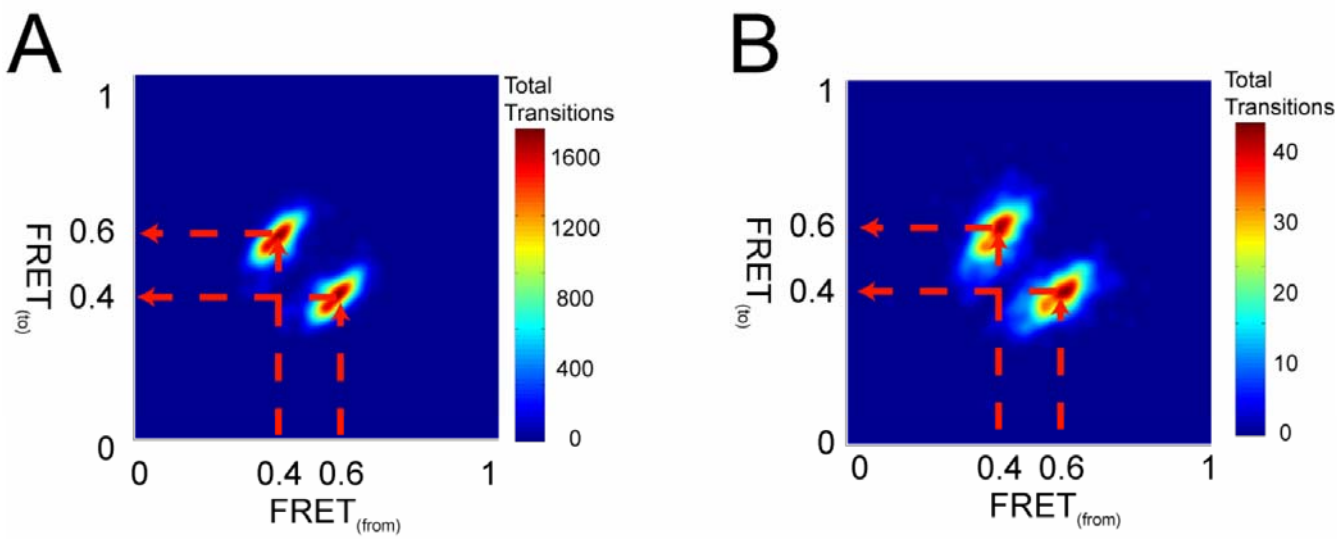


Figure S1. Transition density plot for ribosomal complexes containing tRNAfMet (a) and tRNAMet (b). The TDP is constructed by plotting values for each transition based upon the FRET value from which the transition originated (x-axis) and to which FRET value the transition ends (y-axis). The transition paths are indicated by the broken red arrows.

Table 1. Kinetic Rates Measured between 0.56 and 0.40 FRET States

P Site tRNA/A site tRNA	Forward Transitions (k_1)	$k_1(s^{-1})$	Reverse Transitions (k_{-1})	$k_{-1}(s^{-1})$	Transitlons per Trace
$tRNA^{fMet}$/Vacant	9906	0.51 ± 0.03	9902	0.21 ± 0.03	35
$tRNA^{fMet}$/N-Ac-Phe-$tRNA^{Phe}$	9256	0.27 ± 0.08	9195	0.19 ± 0.02	30
$tRNA^{Met}$/Vacant	3256	0.49 ± 0.12	3242	0.09 ± 0.03	17

Results of fitting FRET time trajectories with the HMM algorithm. Each data set was divided into three and analyzed separately. The reported number is an average from each of the three data sets with the standard deviation.