

Table S1. Testing the Effect on –1PRF of the aa-tRNAs Decoding mRNA Downstream of the Frameshifting Site, Related to Figure 1

Ternary complex concentration, μM		Rate, s^{-1} ^a		–1PRF, % ^b
Val	Phe	Val, –1-frame	Phe, 0-frame	
1.5	1.5	0.3 ± 0.05	0.09 ± 0.01	75 ± 10
0	1.5	–	0.07 ± 0.02	–
1.5	0	0.2 ± 0.05	–	–
1	4	0.5 ± 0.06	0.06 ± 0.02	80 ± 10
4	1	0.3 ± 0.05	0.05 ± 0.01	80 ± 10

^a Rates of incorporation of Val and Phe into peptide, as determined by global fitting of kinetic data similar to those shown in Figure 1D-H.

^b Average –1PRF efficiency calculated from end levels of Val and Phe incorporation.

Table S2. Testing the Functionality of Fluorescence-Labeled Ribosome Complexes, Related to Figures 2 and 4

Step	Rates, s ⁻¹		
	Wild-type ^c	L12/EF-G ^d	S13/tRNA ^{Leu e}
Tyr ^a	9.9 ± 0.5	9.5 ± 0.4	15.8 ± 0.6
Tl _{MY} ^b	2.7 ± 0.1	2.0 ± 0.1	3.1 ± 0.1
Leu ^a	13.6 ± 3.2	14.9 ± 2.5	9.8 ± 1.2
Lys ^a	5.1 ± 0.4	6.0 ± 0.7	5.7 ± 0.3
Val ^a	0.2 ± 0.05	0.5 ± 0.1	0.5 ± 0.03
Phe ^a	5.3 ± 0.6	4.0 ± 0.5	3.3 ± 0.2

^a Rates of incorporation of various amino acids into peptide on *-/-* mRNA.

^b Rate of the 1st translocation event (Tl_{MY}; translocation of MY-tRNA^{Tyr} from the A to P site).

^c Unmodified ribosomes and tRNAs.

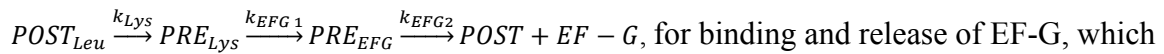
^d Ribosomes reconstituted with L12(A1x) and EF-G(QSY).

^e Ribosome complexes contained S13(AttoQ) and tRNA^{Leu}(Flu).

Table S3. Fitting of Fluorescence Changes upon MYLK-tRNA^{Lys} Translocation by Numerical Integration, Related to Figures 2 and 4

Observables	Rate, s ⁻¹			
	-/- mRNA, +K	-/- mRNA, +K/V/F	+/+ mRNA, +K	+/+ mRNA, +K/V/F
FRET L12-EF-G				
k_{Lys}	4.8 ^a		4.8 ^a	
k_{EFG1} (EF-G binding)	>10 ^b		4.8 ± 0.1	
k_{EFG2} (EF-G release)	3.0 ± 0.2		0.2 ± 0.01	
ΔF of tRNA ^{Leu}				
k_{Lys}	4.9 ^a	4.9 ^a	4.9 ^a	4.9 ^a
k_{app1}	- ^c	- ^c	>10 ^{b,d}	>10 ^{b,d}
k_{app2}	9.0 ± 0.2 ^e	9.8 ± 0.6 ^e	0.9 ± 0.1 ^f	0.9 ± 0.1 ^f
k_{app3}	3.0 ± 0.2 ^g	3.3 ± 0.1 ^g		0.2 ± 0.02 ^g
FRET S13-tRNA ^{Leu}				
k_{Lys}	5.7 ^a	5.7 ^a	5.7 ^a	5.7 ^a
k_{app1}	9.0	9.0	0.9	0.9
k_{app2}	3.4 ± 0.2 ^g	3.3 ± 0.3 ^g	0.25 ± 0.03 ^g	0.21 ± 0.1 ^g

Apparent rate constants were determined by numerical integration using the following reaction sequences:



corresponded to the minimum number of steps required for fitting, and

$POST_{Leu} \xrightarrow{k_{Lys}} PRE_{Lys} \xrightarrow{k_{EFG1}} PRE_{EFG} \xrightarrow{k_{app1}} POST1 \xrightarrow{k_{app2}} POST2 \xrightarrow{k_{app3}} POST3$, for tRNA translocation with the minimum number of k_{app} terms required for fitting, two or three as appropriate. Fitting to a smaller number of steps did not yield satisfactory fits.

^aThe values for k_{Lys} were determined by quench flow (Table S3) and used as a fixed parameter for fitting.

^b As EF-G binding precedes translocation, EF-G recruitment step was included in fitting the FRET changes for $+/+$ mRNA (4.8 s^{-1} , taken as a fixed parameter in fitting). For the $-/-$ mRNA this was not necessary, because EF-G recruitment was relatively rapid.

^c-, not observed.

^d The lowest estimate compatible with the fit; the step represents the transition from PRE_{EF-G} to $POST1$ (see main text).

^e Represent translocation from PRE_{EF-G} to $POST1/2$ on $-/-$ mRNA; the rate corresponding to k_{app1} observed for the $+/+$ mRNA was not resolved, as the rates, $>10 \text{ s}^{-1}$ and $9-10 \text{ s}^{-1}$, were too similar. The steps are distinguished based on the differences in the fluorescence change of $tRNA^{Leu}$ (Fig. 4H and I, see also Discussion).

^f Transition $POST1$ to $POST2$ for $+/+$ mRNAs; represents the sum of the rate constants at the kinetic partitioning branch (Figure 5).

^g Transition from $POST2$ to $POST3$, which entails dissociation of EF-G, $tRNA^{Leu}$, and the backward rotation of the 30S head.