

Kinetics of programmed and spontaneous ribosome sliding along the mRNA

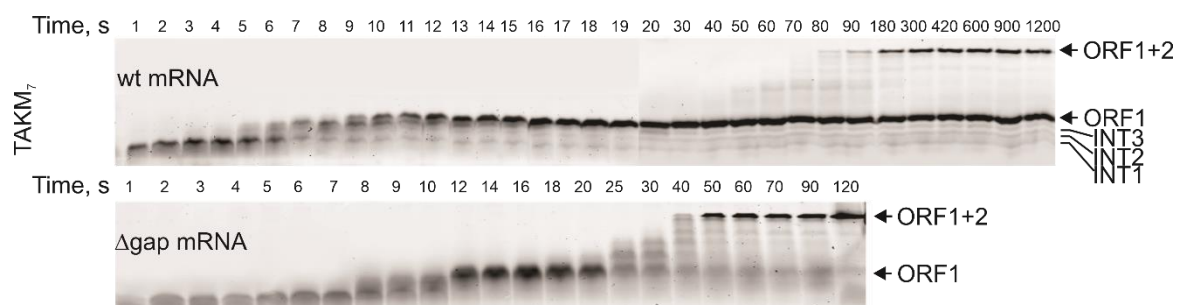
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Supplementary Table 1. Kinetics of *gene 60* translation in HiFi and TAKM₇ buffers

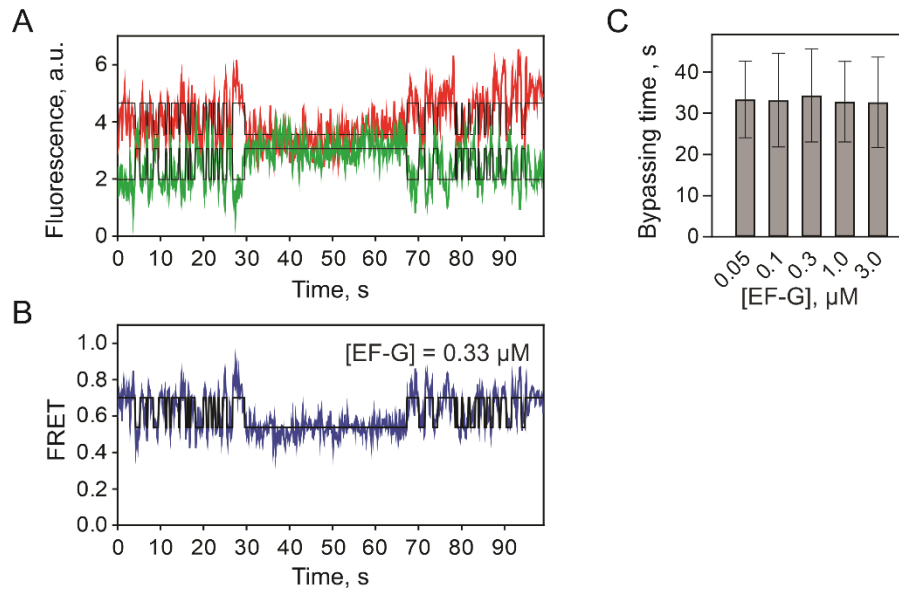
Translation time courses were evaluated by fitting to a delay (t_{delay}) followed by exponential increase in product formation (t_{exp}), which together yield the total synthesis time (t_{total}). The t_{gap} value is calculated from t_{total} (ORF1+ORF2) – t_{total} (Δgap). The average elongation rate is calculated as length of peptide in aa / t_{total} .

mRNA	t_{delay} , S	t_{exp} , S	t_{total} , S	t_{gap} , S	k_{el} , aa/s
ORF1, HiFi	3.2 ± 0.2	3.9 ± 1.5	7.1 ± 0.4		6.3 ± 0.3
ORF1+ORF2, HiFi	44.3 ± 1.5	32.0 ± 2.7	76.3 ± 3.1	44.6 ± 6.2	2.1 ± 0.1
Δgap , HiFi	17.8 ± 3.3	13.9 ± 4.3	31.7 ± 5.4		5.0 ± 0.6
ORF1, TAKM ₇	6.9 ± 0.3	8.8 ± 2.1	15.7 ± 2.1		2.9 ± 0.4
ORF1+ORF2, TAKM ₇	63.9 ± 2.3	88.5 ± 8.0	152.4 ± 8.3	99.8 ± 8.5	1.0 ± 0.1
Δgap , TAKM ₇	38.6 ± 0.6	14.0 ± 1.6	52.6 ± 1.7		3.0 ± 0.1



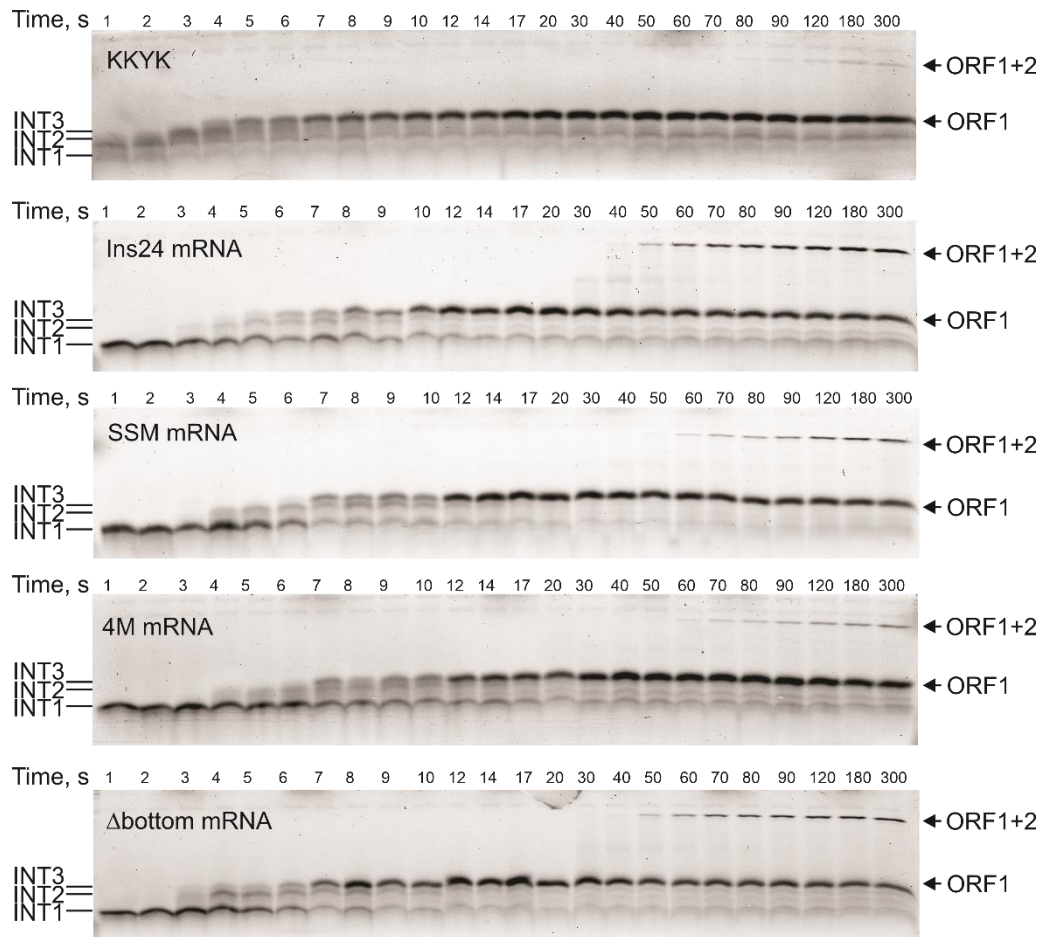
Supplementary Fig. 1. Bypassing in TAKM₇.

Time courses of translation for the full-length wt and Δgap mRNAs in TAKM₇. Translation products were separated by SDS-PAGE and detected using the fluorescent reporter (BodipyFL) attached to the N-terminal Met in the peptide. Translation of ORF1 is terminated at the UAG stop (46 aa-long ORF1 product); ORF1+2 is the product of bypassing and translation to the end of the ORF2 (160 aa). Δgap is the construct containing ORF1+ORF2 without the proximal stop codon and the non-coding gap.



Supplementary Fig 2. Determination of the bypassing time by smFRET.

- Representative single-molecule fluorescence intensity traces showing donor (green) and acceptor (red) fluctuations between non-rotated and rotated states of the ribosome.
- smFRET calculated from the time courses shown in A. The trace shows rapid transitions between FRET 0.7 (non-rotated state) and 0.5 (rotated state) representing successive elongation cycles. The long-lived FRET 0.5 state corresponds to bypassing, which includes take-off, sliding and landing. After bypassing, the ribosome resumes rapid translation, as indicated by rapid fluctuations. Black solid line shows the HMM fit of the data.
- Bypassing time at different EF-G concentrations shown as mean value with error bars representing standard deviation from the following number of traces: $N = 61$ at $[EF-G] = 0.05 \mu M$, $N = 181$ at $[EF-G] = 0.1 \mu M$, $N = 305$ at $[EF-G] = 0.3 \mu M$, $N = 241$ at $[EF-G] = 1.0 \mu M$, and $N = 48$ at $[EF-G] = 3 \mu M$.



Supplementary Fig. 3. Time courses of ORF1 and ORF1+2 synthesis on mutant mRNA constructs shown in Fig. 3A.