

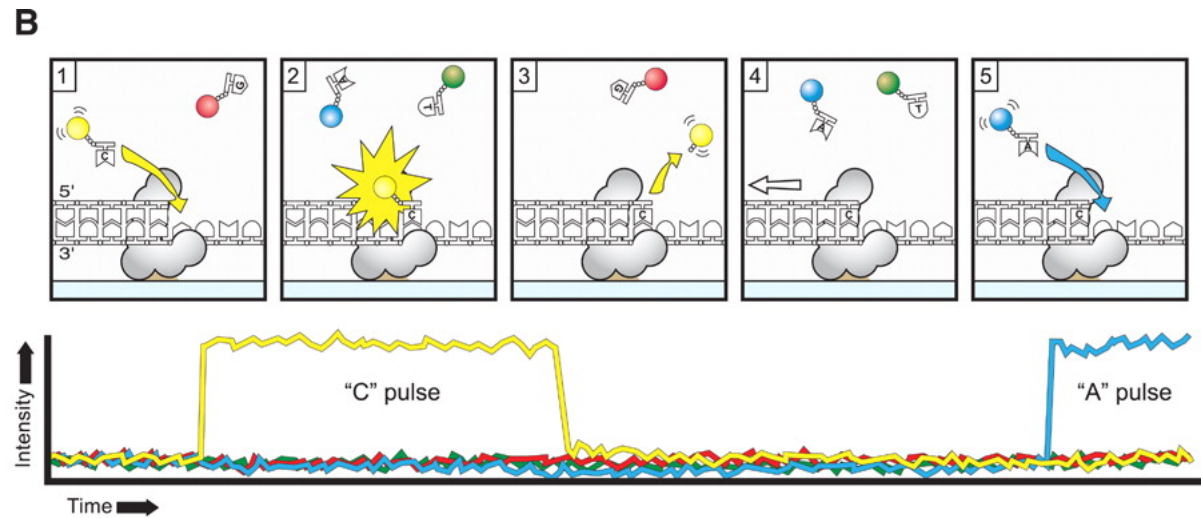
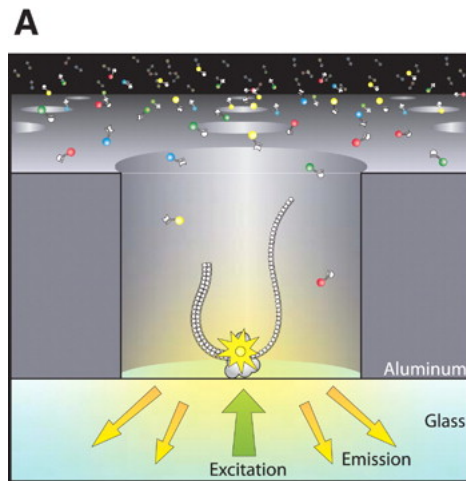
Real-time tRNA transit on single translating ribosomes at codon resolution

Mikk Eelmets

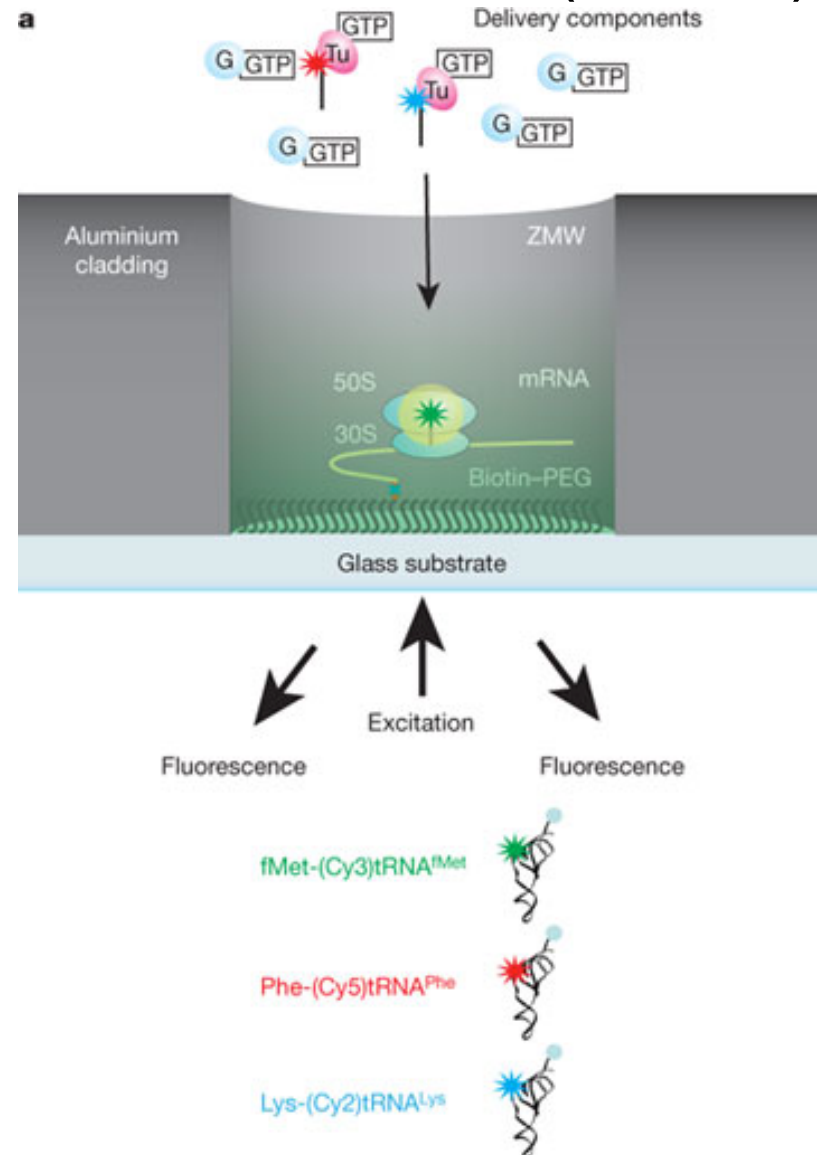
Journal Club

21.02.2011

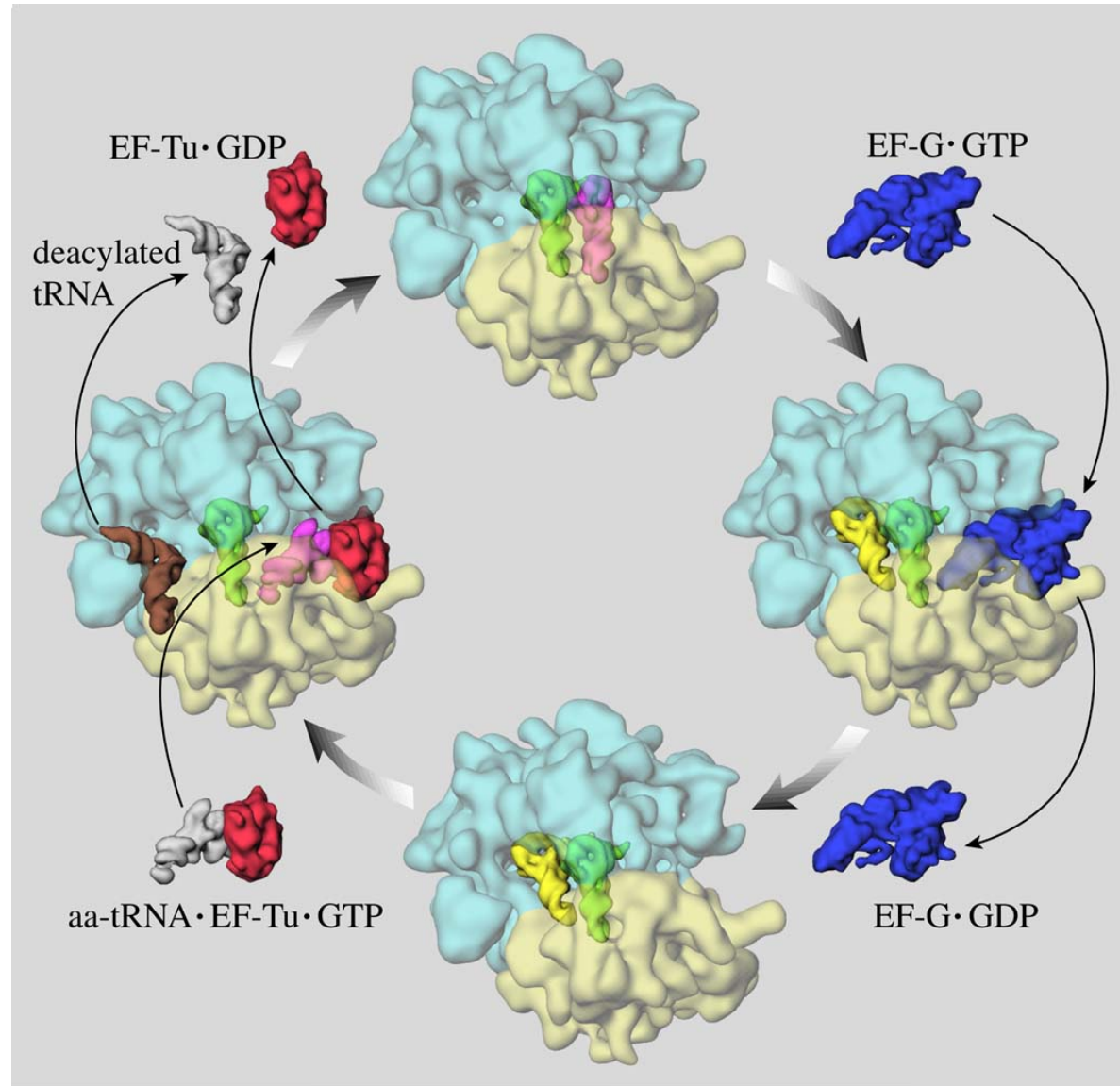
Single Molecule Real Time (SMRT) technology



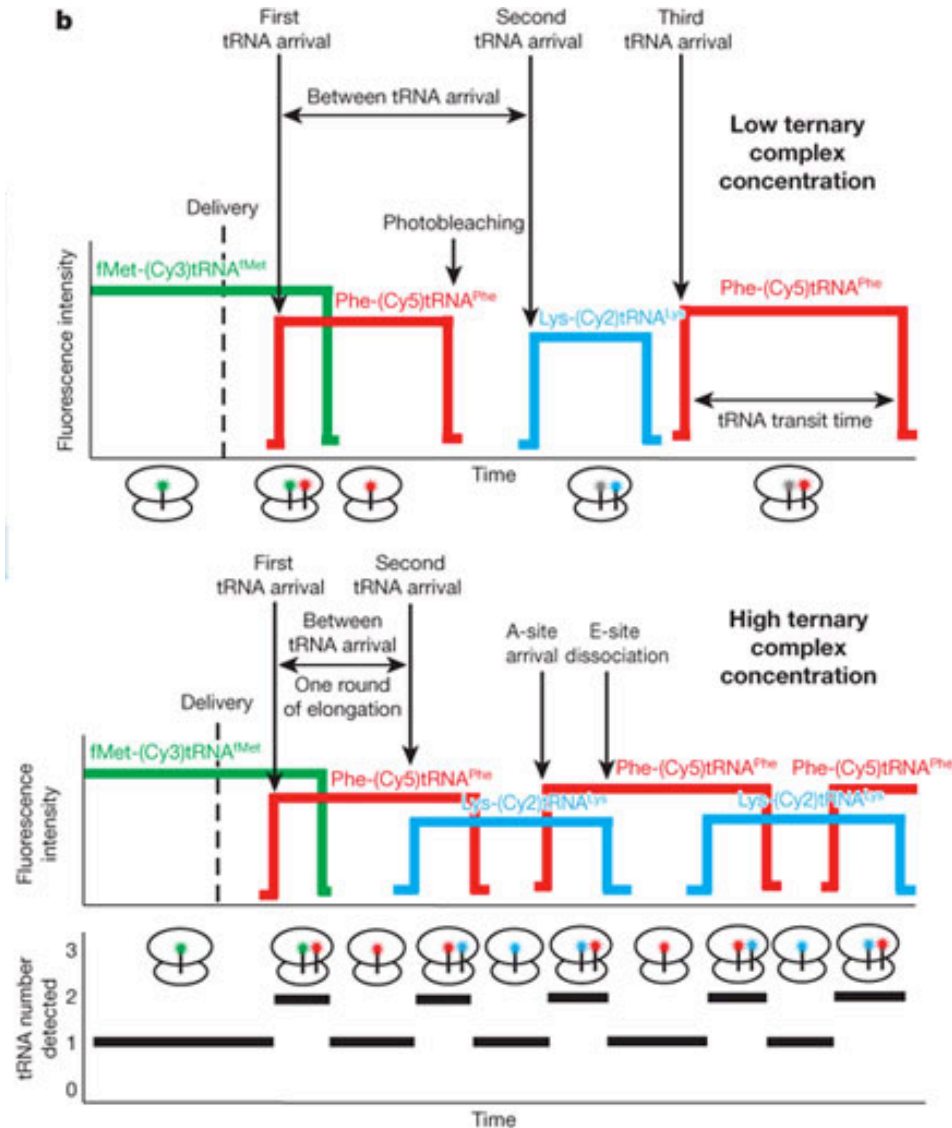
Single Molecule Real Time (SMRT) technology



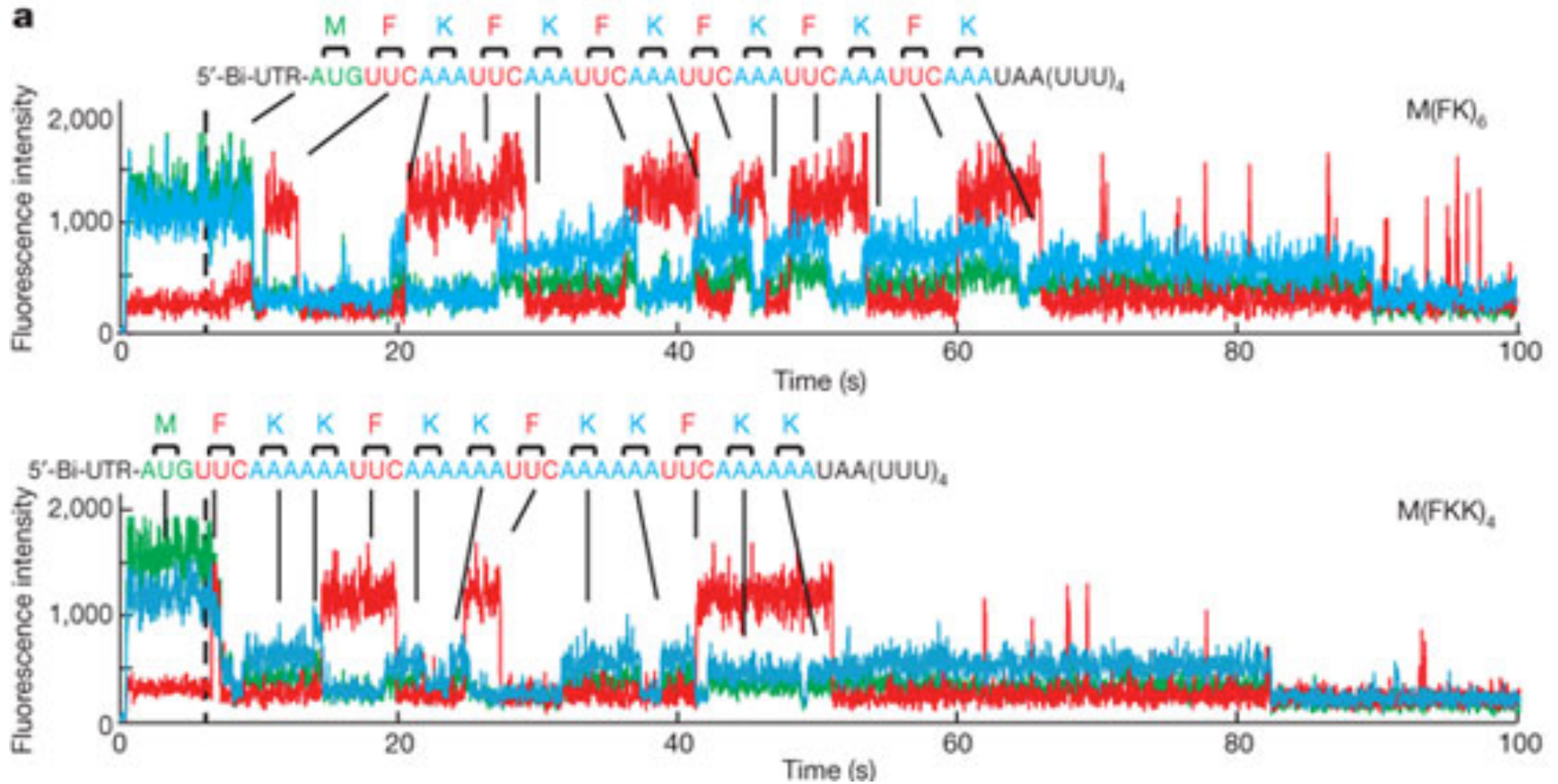
Protein synthesis



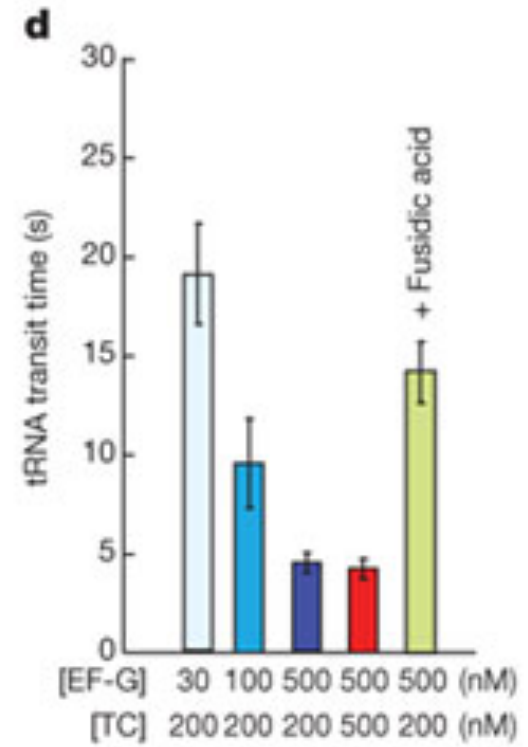
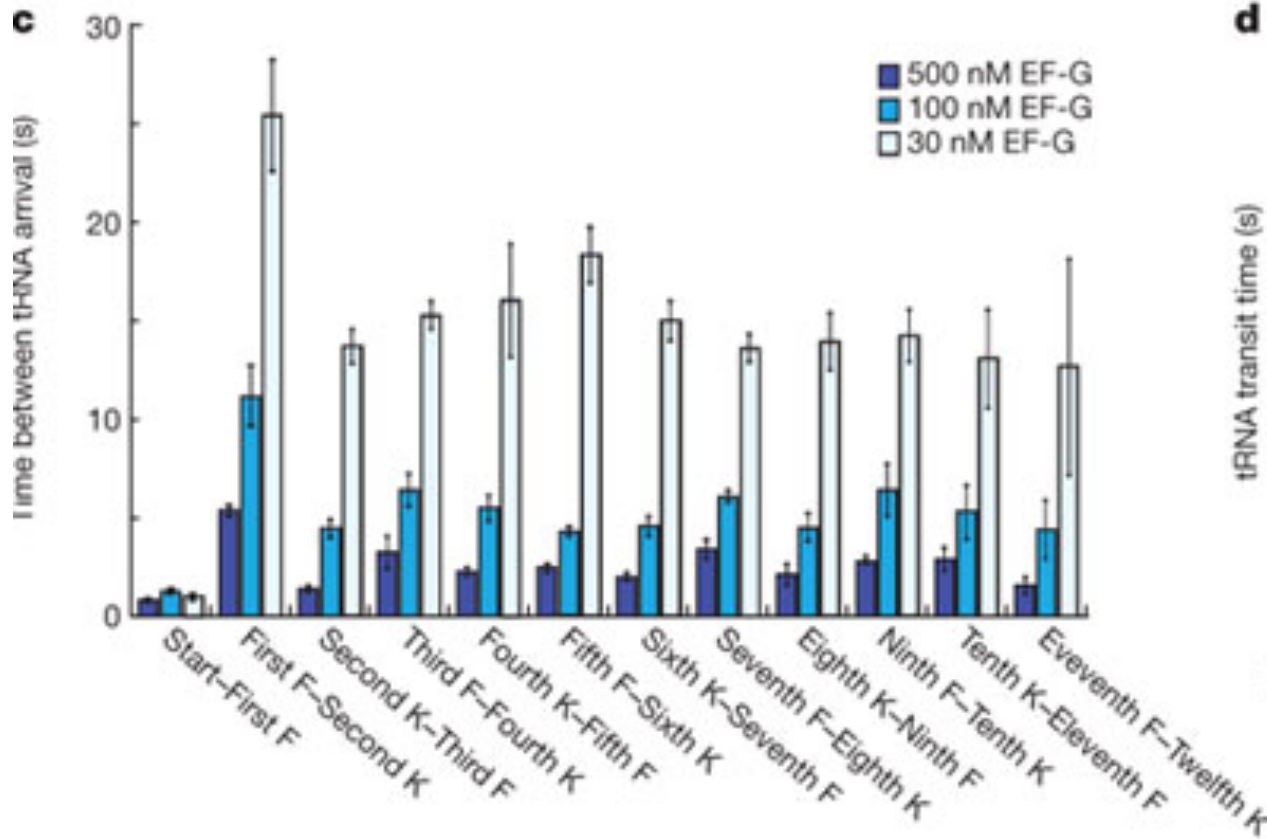
Single Molecule Real Time (SMRT) technology



Monitoring translation in real time

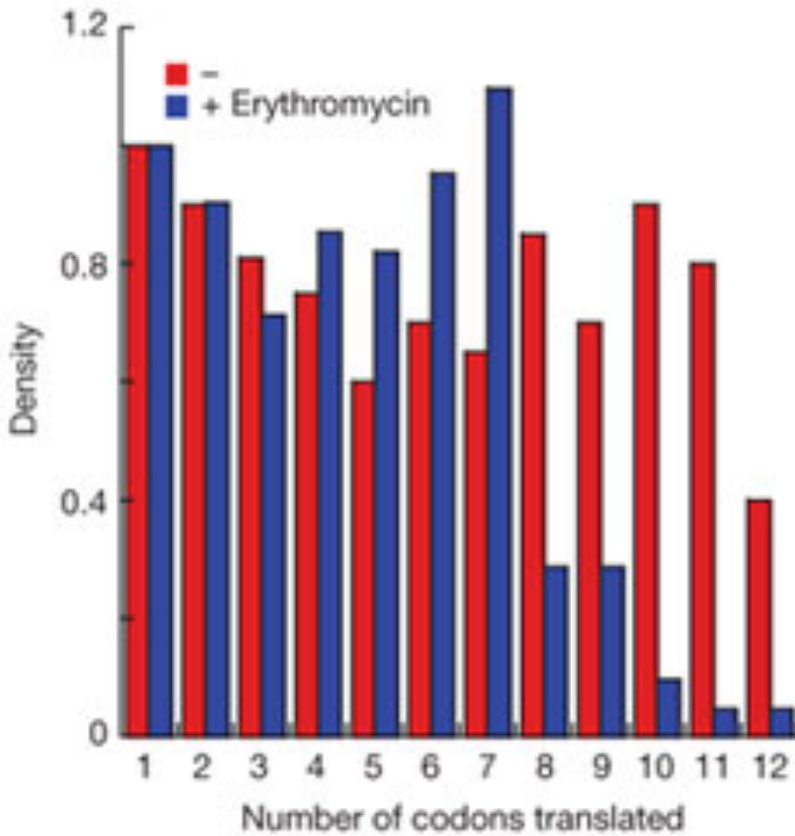


Monitoring translation in real time

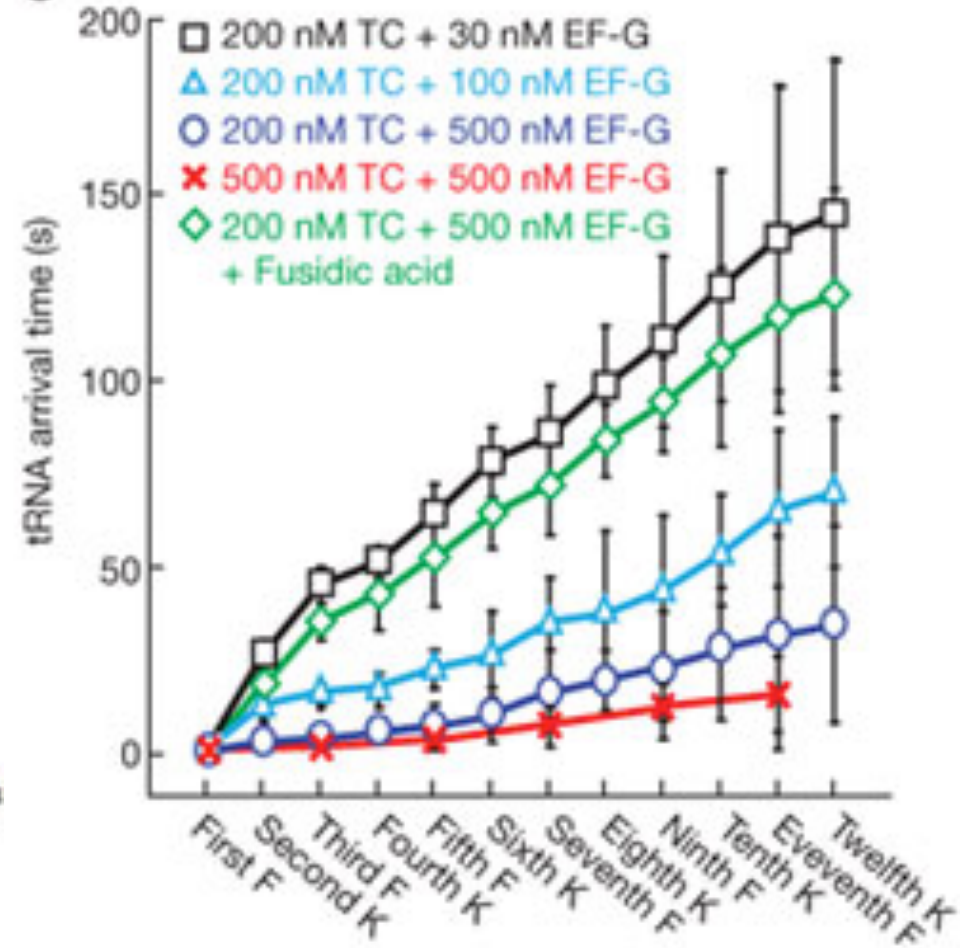


Monitoring translation in real time

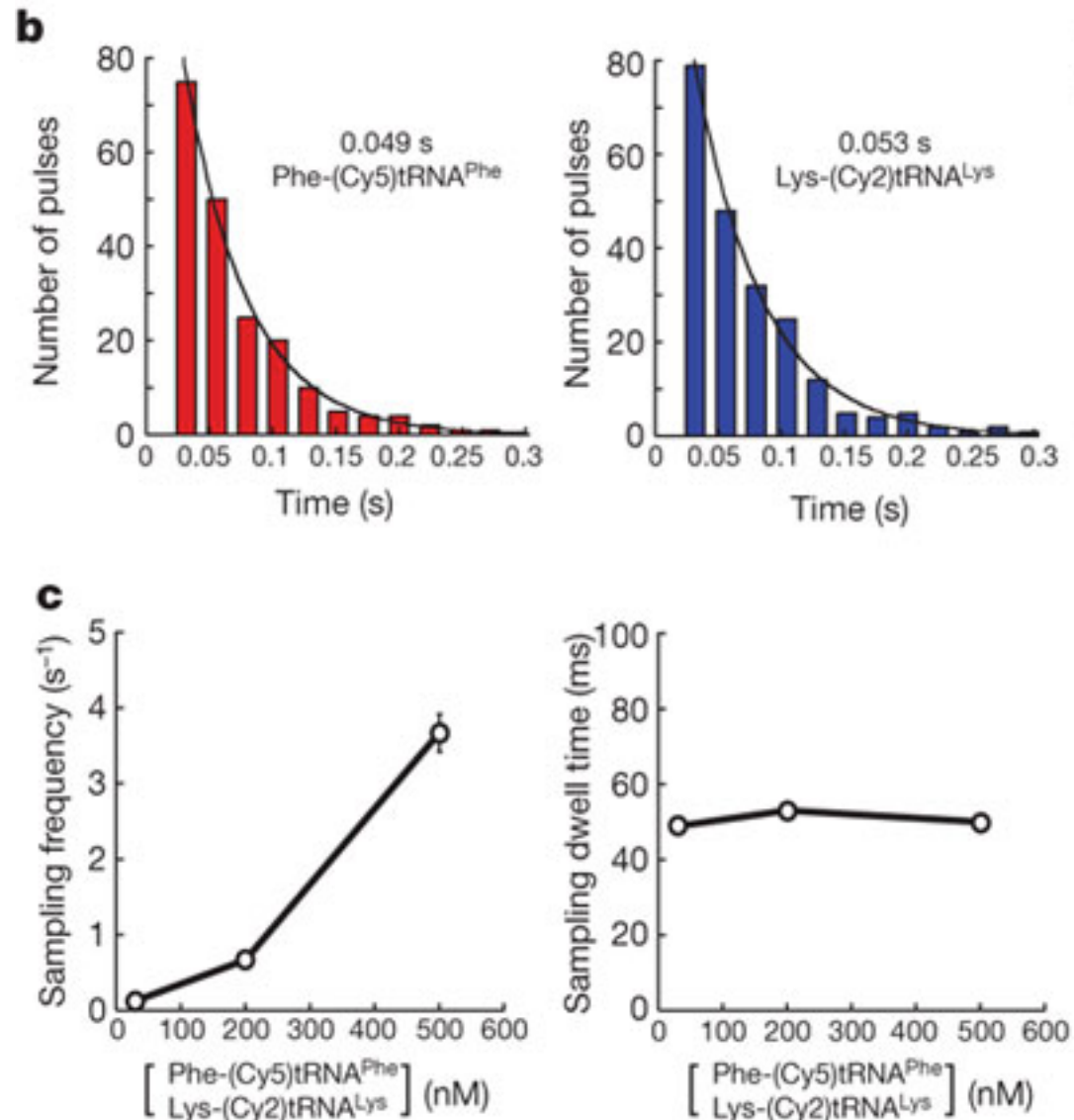
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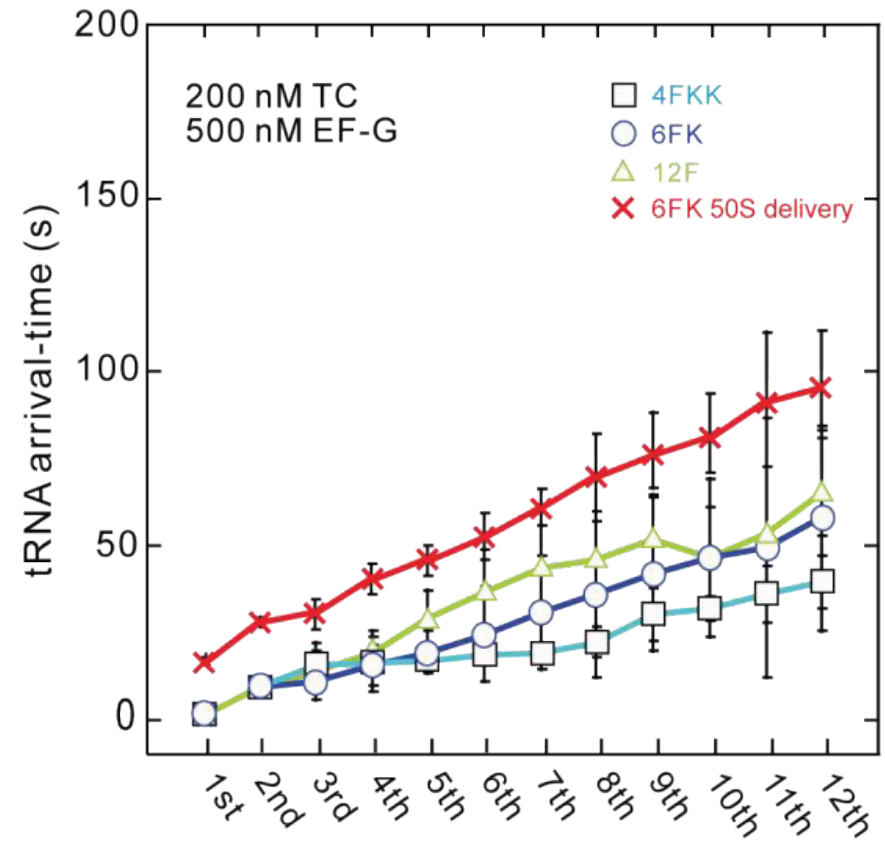
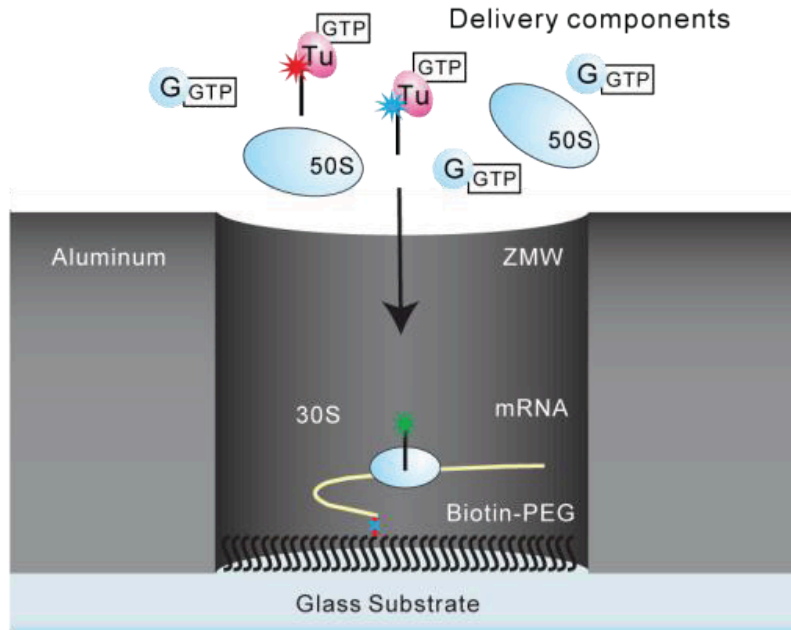
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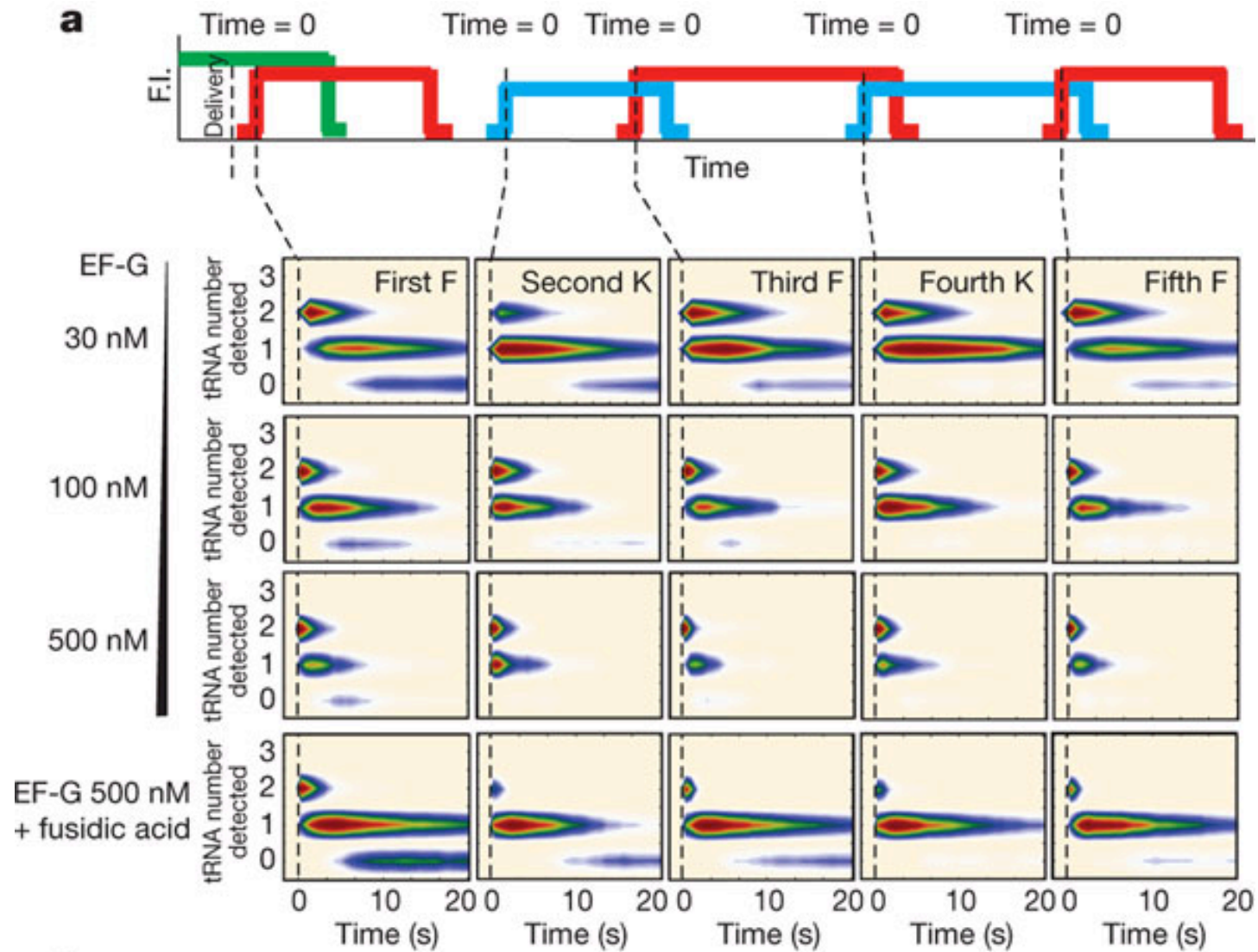
Stop-codon sampling



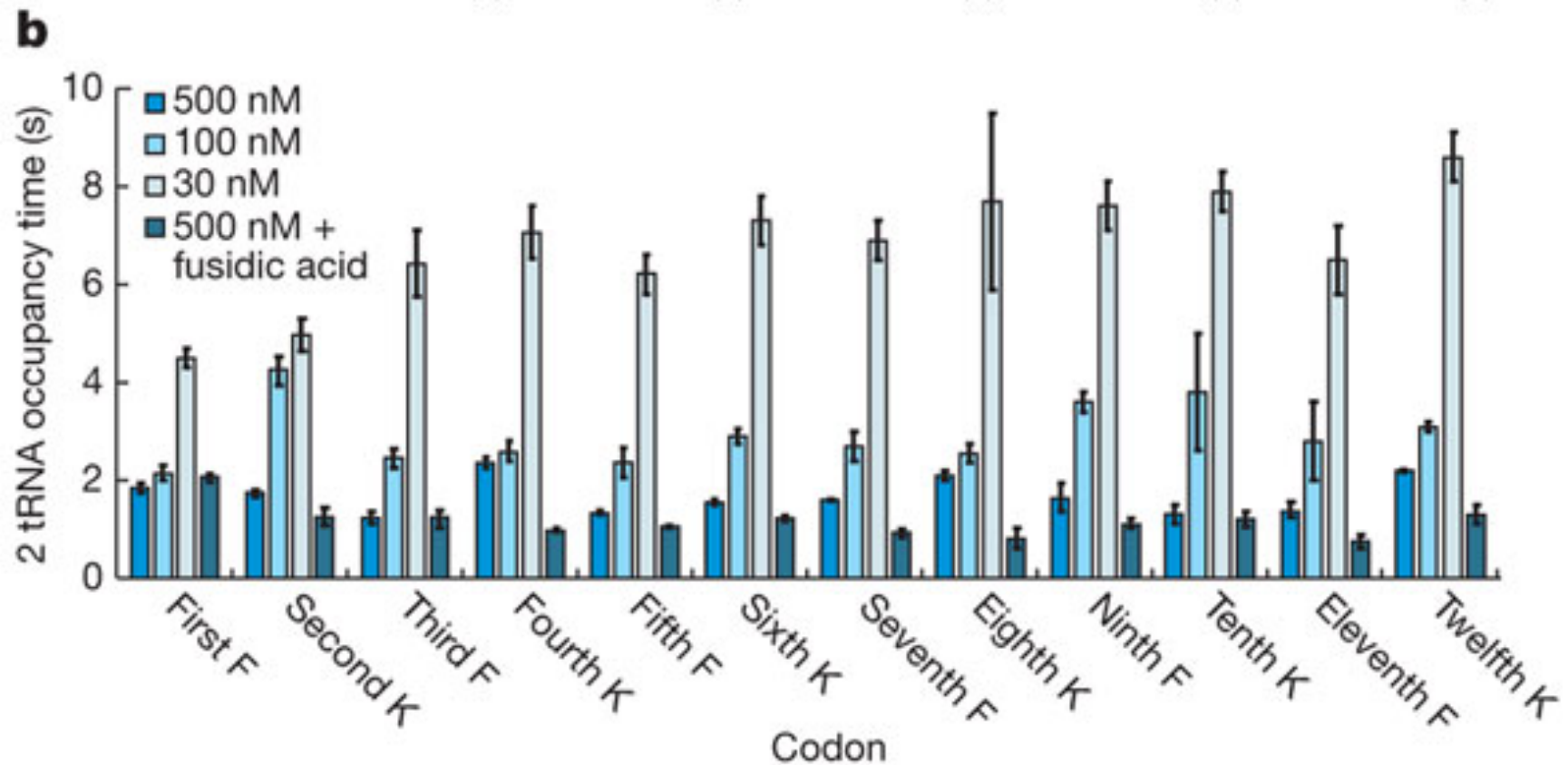
50S subunit joining and translocation time



Dynamic tRNA occupancy during translation



Dynamic tRNA occupancy during translation



Conclusion

- Translation can be observed in real time using single ribosomes immobilized in ZMWs
- Used antibiotics interfere translation as predicted by their mechanism
- tRNA releases rapidly from E site after translation has occurred (uncorrelated with arrival of next tRNA). Three tRNAs are rarely observed on translating ribosomes.

Reference

Uemura S, Aitken CE, Korlach J, Flusberg BA, Turner SW, Puglisi JD.

" Real-time tRNA transit on single translating ribosomes at codon resolution."

Nature. 2010 Apr 15;464(7291):1012-17

THANK YOU

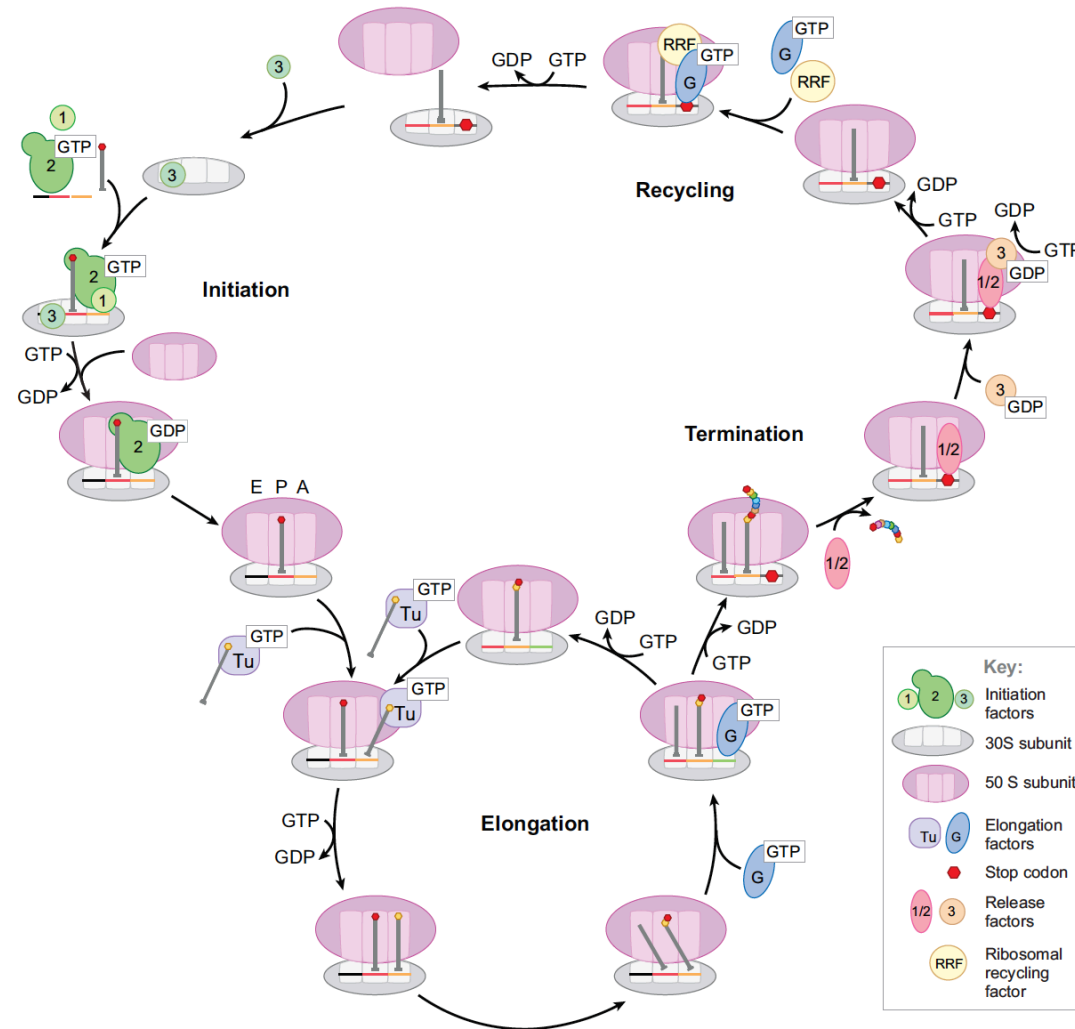


Figure 1

The prokaryotic translation cycle. Shown is the current model, which has been derived from biochemical and biophysical studies. Initiation, mediated by initiation factors 1, 2, and 3 (green-shaded circles), culminates in the joining of 30S (gray) and 50S (purple) subunits on the mRNA message primed with initiator tRNA (gray line with red circle) in the P site. This complex, aided by the elongation factors Tu and G (blue-shaded circles), subsequently undergoes multiple rounds of elongation. Termination, under the control of release factors 1, 2 and 3 (red-shaded circles), frees the newly synthesized polypeptide upon recognition of the stop codon. Ribosomal recycling factor (yellow circle) and elongation factor G then prepare the translational machinery for subsequent initiation events. Abbreviations: A, ribosomal A site; E, ribosomal E site; G, elongation factor G; P, ribosomal P site; RRF, ribosome recycling factor; Tu, elongation factor Tu.