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ARTICLES

Following translation by single ribosomes one codon at a time

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We have followed individual ribosomes as they translate single messenger RNA hairpins tethered by the ends to optical tweezers. Here we reveal that translation occurs through successive translocation-and-pause cycles. The distribution of pause lengths, with a median of 2.8 s, indicates that at least two rate-determining processes control each pause. Each translocation step measures three bases—one codon—and occurs in less than 0.1 s. Analysis of the times required for translocation reveals, surprisingly, that there are three substeps in each step. Pause lengths, and thus the overall rate of translation, depend on the secondary structure of the mRNA; the applied force destabilizes secondary structure and decreases pause durations, but does not affect translocation times. Translocation and RNA unwinding are strictly coupled ribosomal functions.

Current understanding of the ribosome and the mechanism of translation has been significantly strengthened and expanded by recent advances in crystallography[1–4] and cryo-electron microscopy[5–8]. The ribosome undergoes several dynamical structural changes as it moves relative to the mRNA and transfer RNAs during translation[9–11]. Kinetic experiments have given a quantitative description of some of these dynamics during the main steps of the elongation cycle of protein synthesis[12]. During elongation, the secondary structures present in all mRNAs are disrupted to allow movement of the mRNA through the 30S subunit, and the reading of each codon. This task is aided by the mRNA helicase activity of the ribosome that has been localized to the downstream tunnel of the 30S subunit[13]. Moreover, interactions of mRNA pseudoknots or hairpins with the helicase region of the ribosome can shift the reading frame of the mRNA to the −1 frame, and play an important role in regulating gene expression in retroviruses[14–16].

It is extremely difficult to follow the steps of ribosomes during translational elongation using ensemble methods, because the dynamics of individual ribosomes are stochastic[17,18] and it is impossible to synchronize their activity. Here, we have used optical tweezers to follow the step-by-step translation of a single hairpin-forming mRNA molecule by a single ribosome. This approach has allowed us to characterize the dynamics of ribosome translation, measuring the time the ribosome spends at each codon, the number of mRNA nucleotides that move through the ribosome in each translocation step, and the time required per step. We have also determined the effects of mRNA structure on step size and rate, and have studied the effects of internal Shine–Dalgarno sequences[19] on translation arrest. These experiments provide a dynamic picture of the movement of a messenger RNA through a ribosome.

In these experiments, we used a single mRNA hairpin with a ribosome stalled at the 5′ end by omission of a required aminoacyl-tRNA; the RNA was attached to two micrometre-sized beads by RNA–DNA handles. One of the beads was held on a micropipette and the other in an optical trap used to measure the changes in distance between the beads (in nanometres) and the forces applied to the hairpin (in piconewtons) (Fig. 1A)[20–22]. Translation is resumed at the single-molecule level by adding a mixture containing the required aminoacyl-tRNAs. During translation, the ribosome opens the hairpin as it moves through the RNA; thus, each base translocated requires the breaking of a base pair, which corresponds to an increase in the end-to-end distance of the mRNA by about 1 nm at the forces involved in these experiments (15–20 pN)[23]. Translation can thus be followed in real time by monitoring these changes in distance.

To establish that translation occurred in a single molecule of RNA held in the optical tweezers apparatus, we used a 60-base-pair (bp) hairpin that contains unique codons where the ribosome can be stalled (S3hp; see a in Fig. 1B). The progress of translation was established by determining the size of the residual hairpin, through its mechanical unfolding (details presented in Supplementary Information and Supplementary Fig. 1). To verify that the change in hairpin size was caused by a stalled ribosome at the corresponding position on the hairpin, we flowed a ribosome-releasing mixture containing puromycin (see Methods) into the reaction chamber. After a few minutes, the hairpin fully refolded, indicating that the ribosome had been released. These control experiments established that an RNA duplex can be unwound by the ribosome as translation proceeds, and that a ribosome can be stably stalled on the RNA at a chosen position.

We also translated the mRNA in bulk in the absence of force (using *Escherichia coli* S100 enzymes, see Supplementary information). The 60-bp S3hp mRNA and its corresponding wild-type ribosomal S3 mRNA were translated completely, but the hairpin RNA was translated approximately 40% slower (see Supplementary Fig. 2). These results show that ribosomes can translate long helices as used in our experiments, even without the aid of force to unfold them.

Following translation in real time

To follow translation by single ribosomes in real time, we designed another 60-bp hairpin construct, VE60hp, whose first 15 codons in the hairpin region encode only valine (Val) and glutamic acid (Glu), preceded by two phenylalanine codons (Phe) to allow ribosome stalling (see b in Fig. 1B). The hairpin with a ribosome stalled at the Phe

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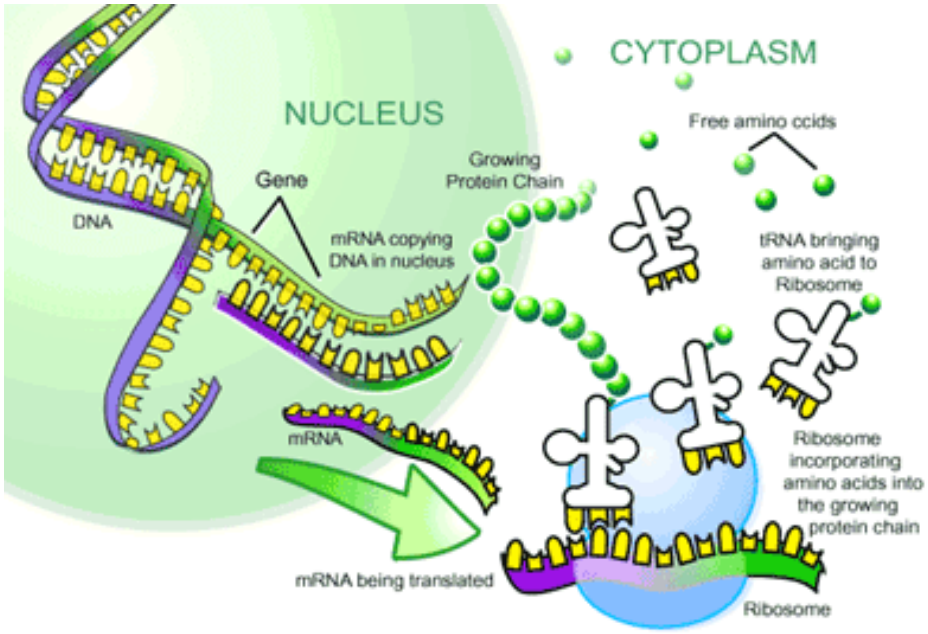
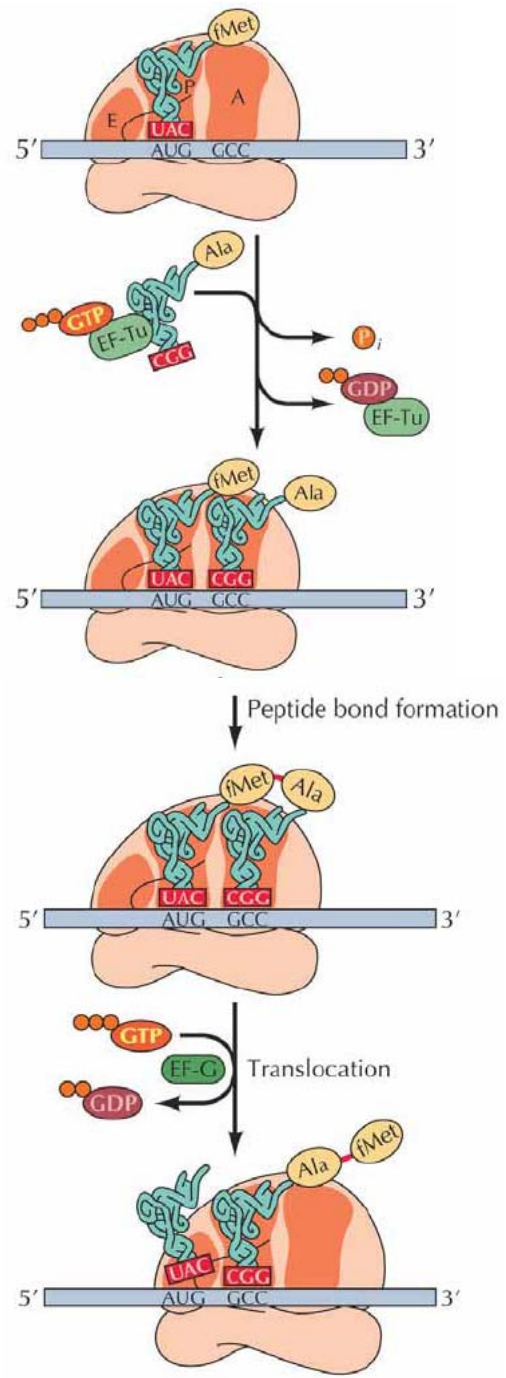
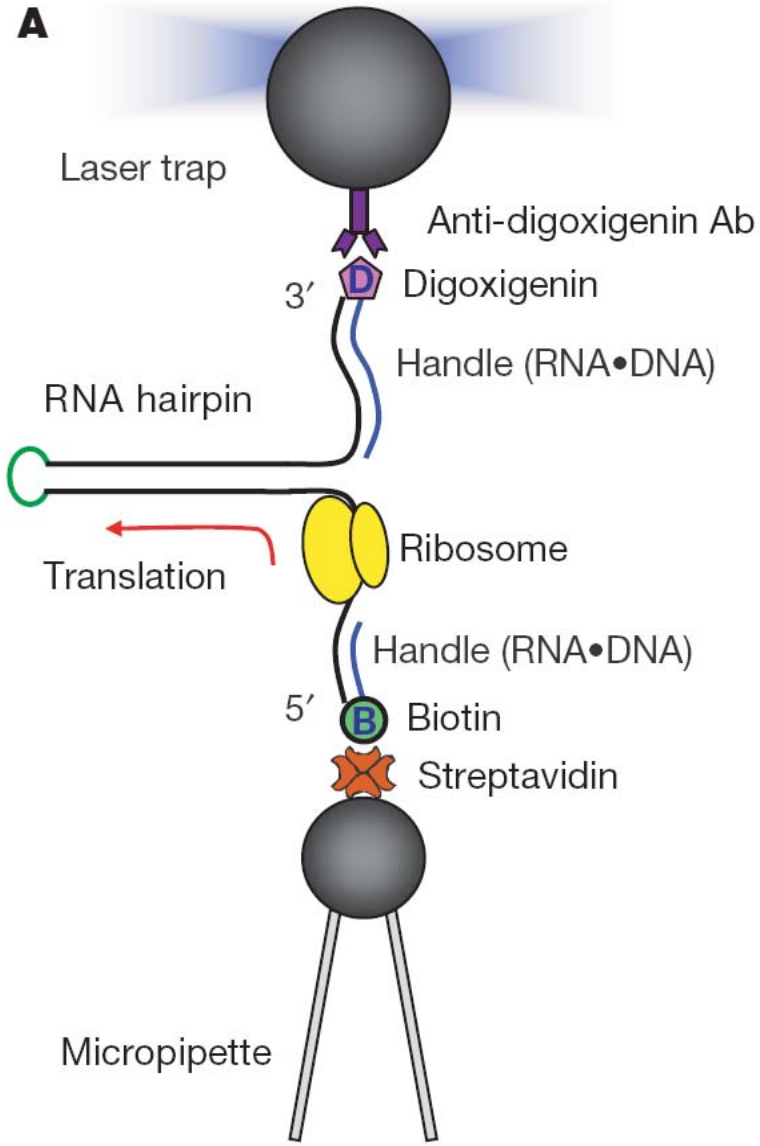
Translation

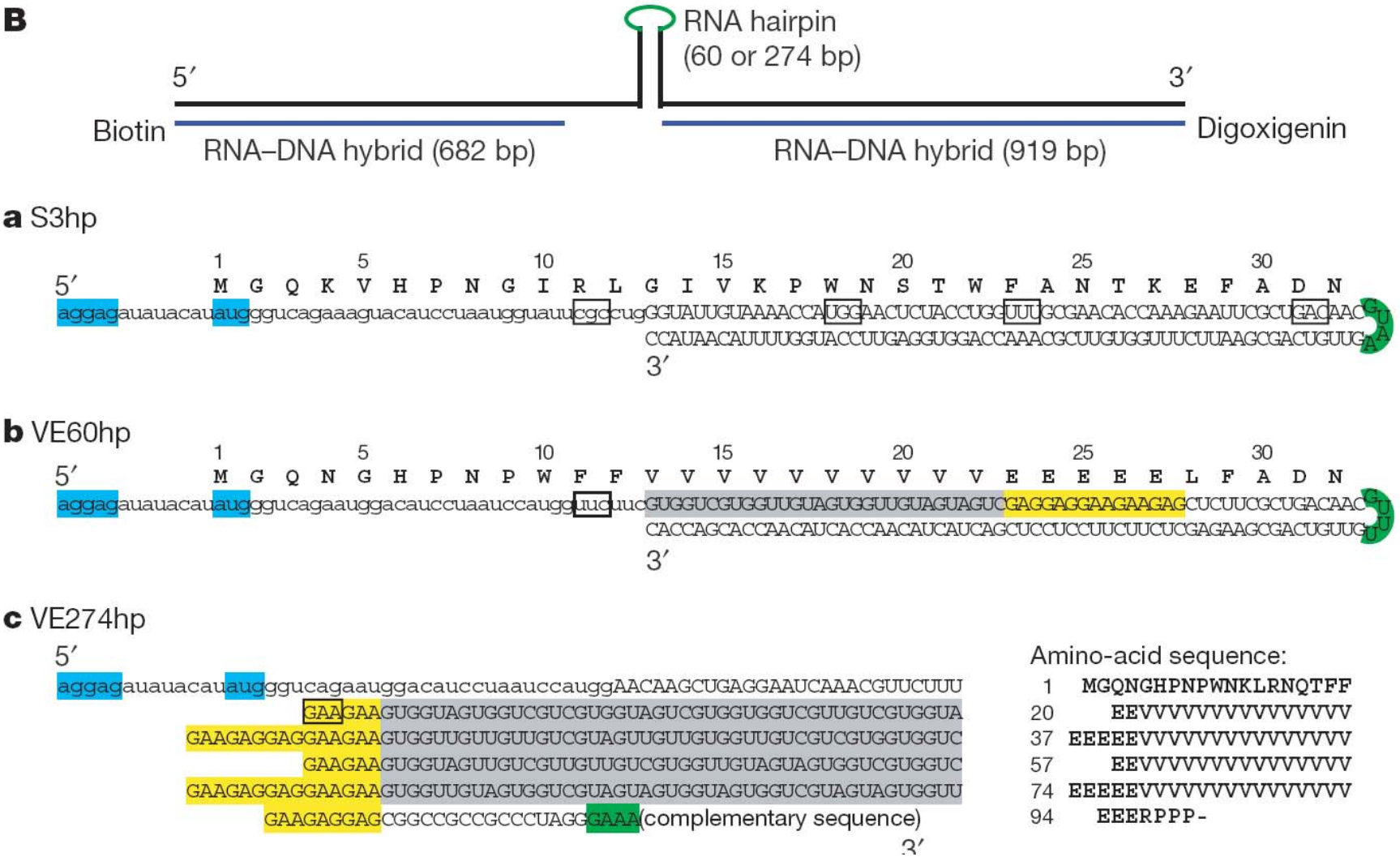




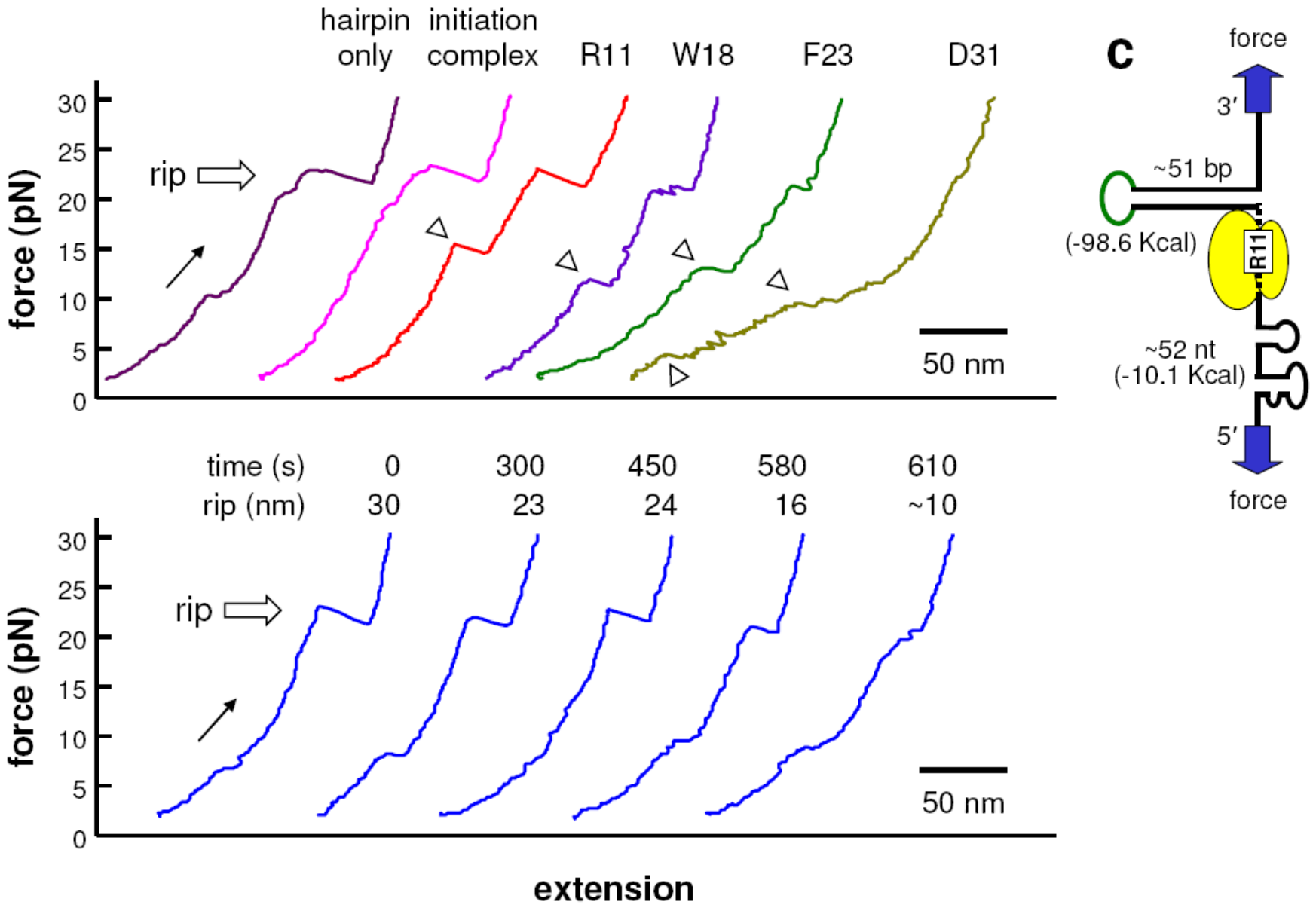
Optical tweezer



mRNA construction

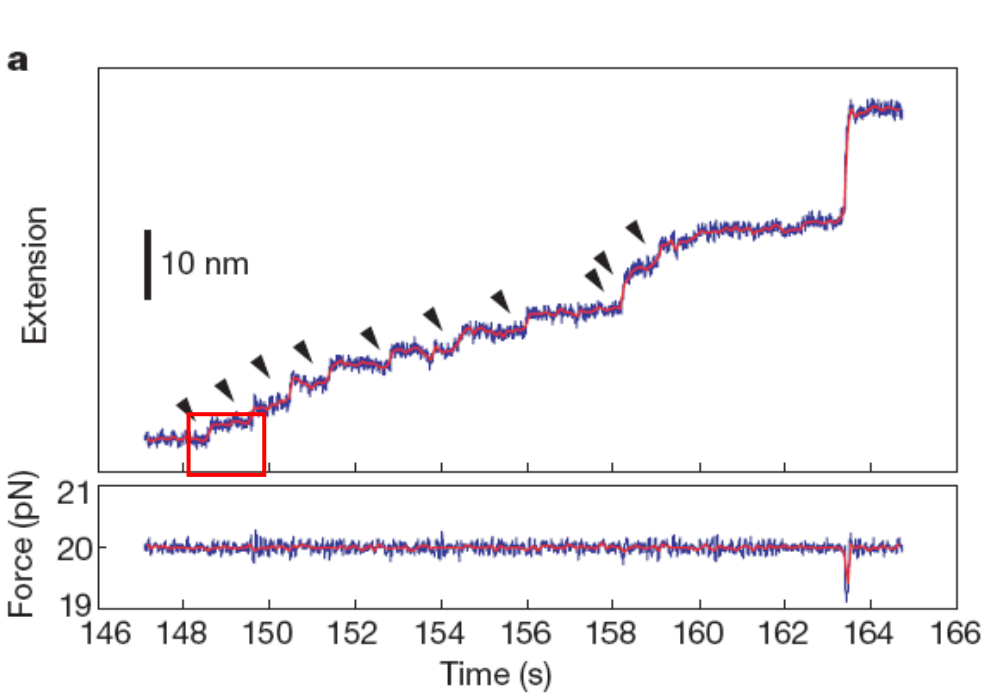


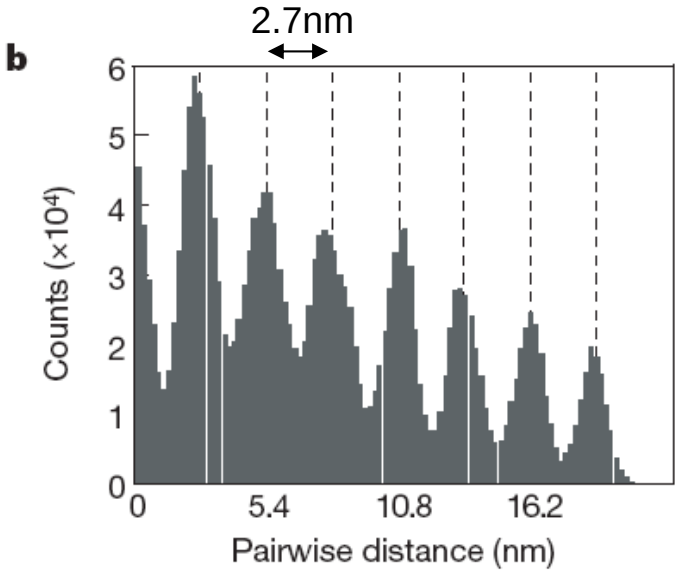
Force extension curve

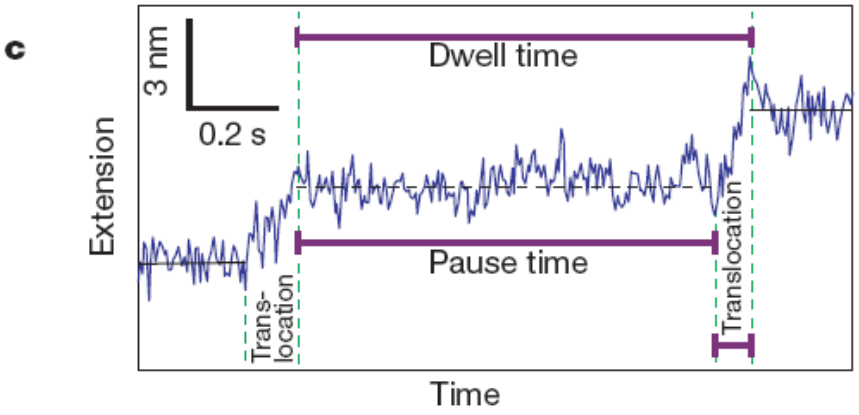


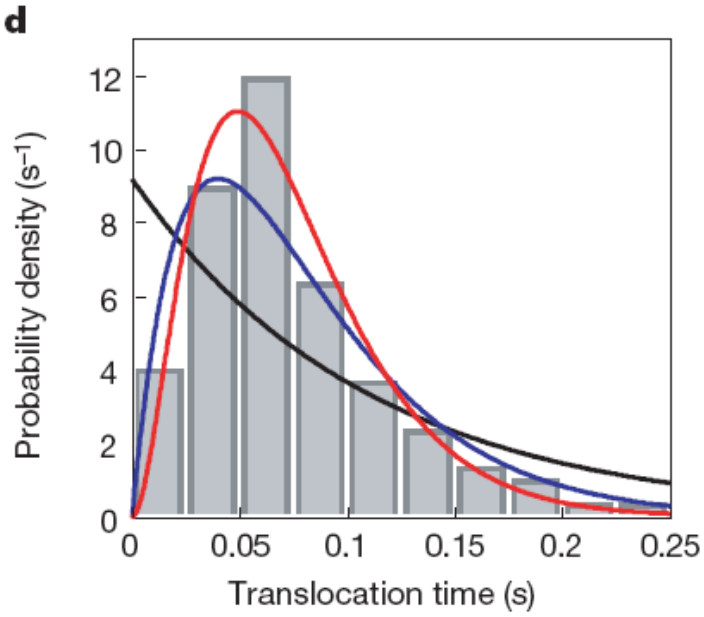
Codon by Codon translation

VE60hp



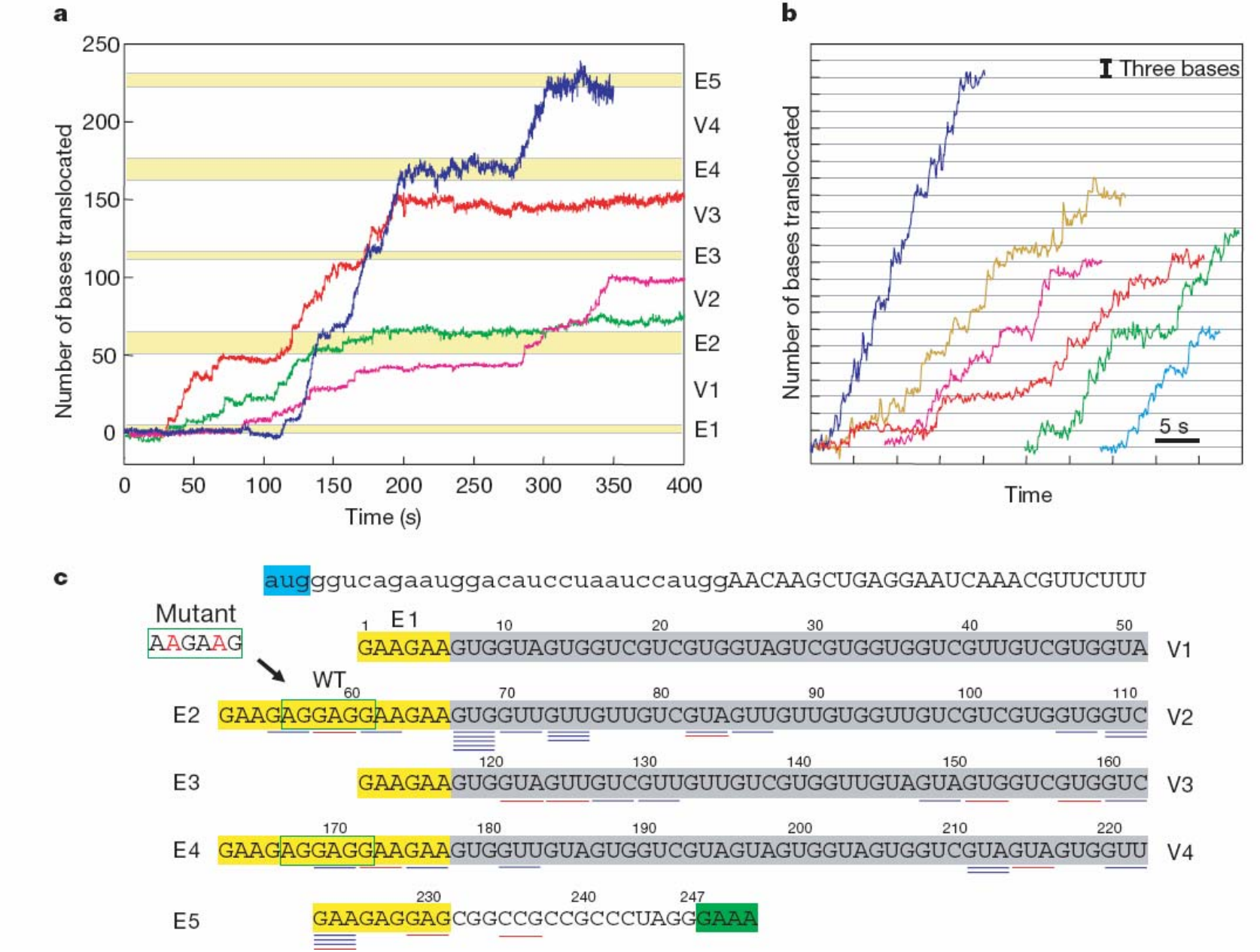




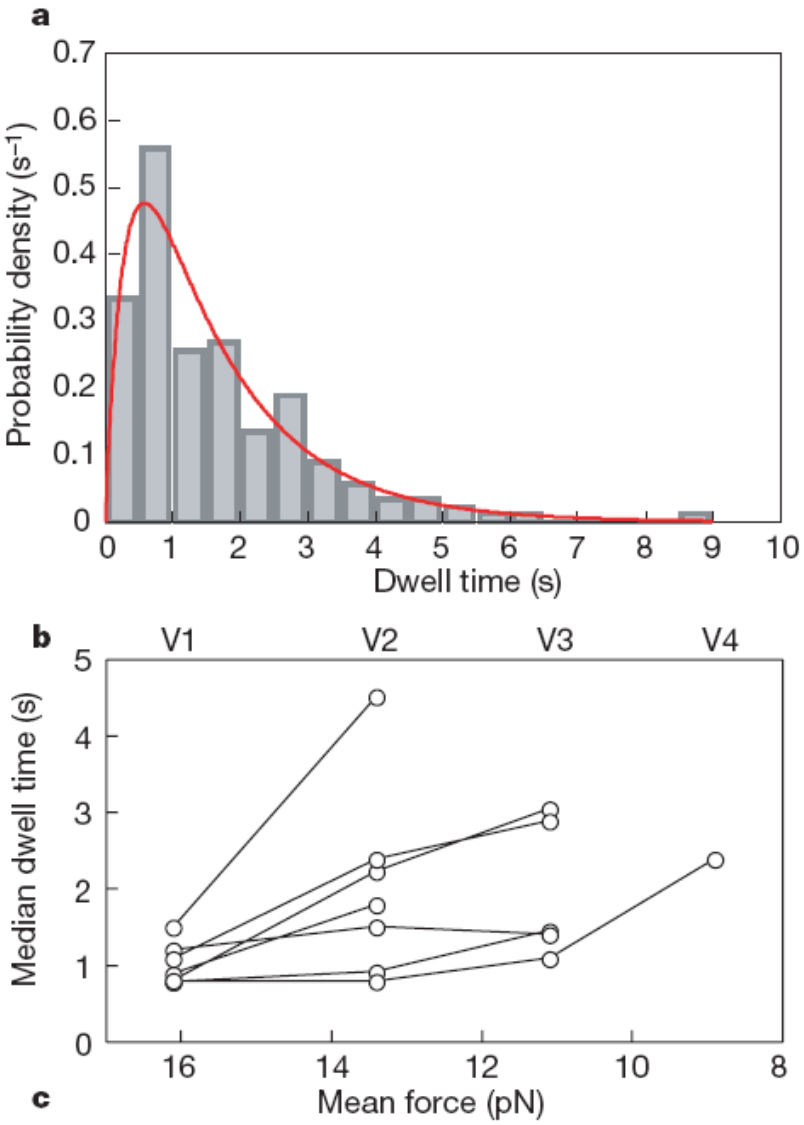


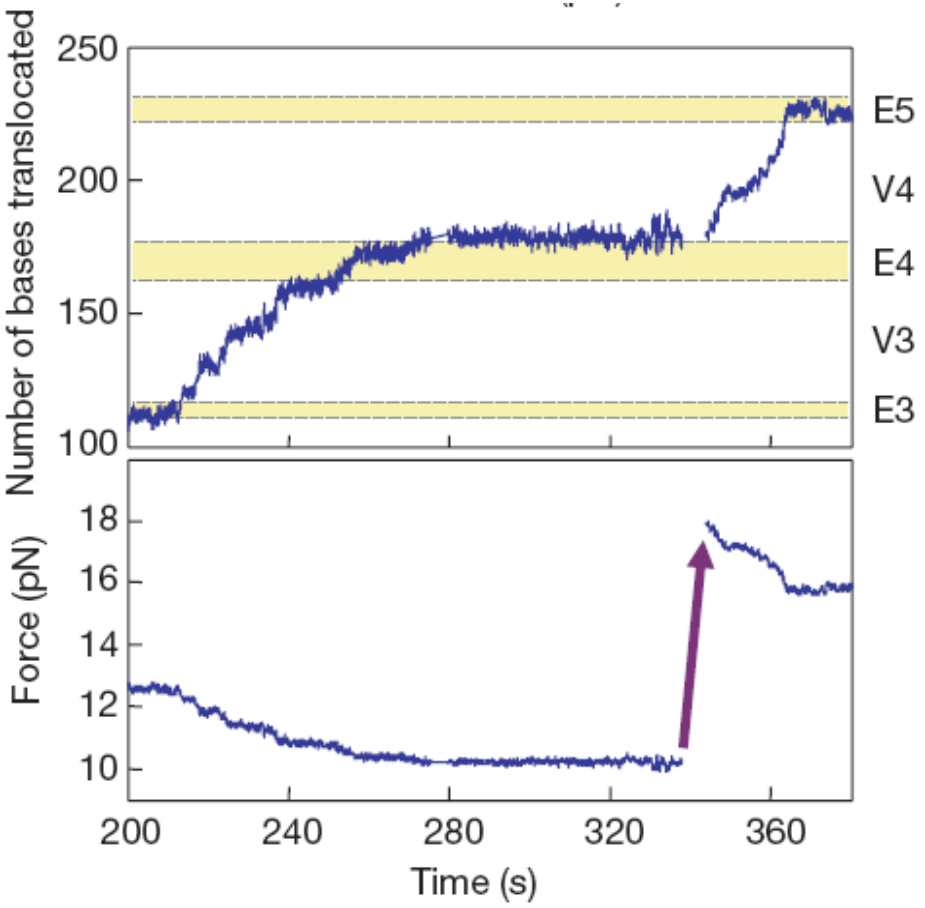
Codon by Codon translation

VE274hp



Pauses and translocations





Conclusions

* Translation occurs through successive translocation-and-pause cycles.

* The pauses consisting of at least two rate-determining processes control each pause.

* Each translocation step measures one codon, possibly consisting three substeps.

* Pause lengths, and thus the overall rate of translation, depend on the secondary structure of the mRNA

* The applied force destabilizes secondary structure and decreases pause durations, but does not affect translocation times.

* Translocation and RNA unwinding are strictly coupled ribosomal functions.