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Breaking the barriers of translation

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Abstract

New data from single-molecule optical trapping methods provide exciting clues as to the underlying mechanism of ribosome translocation along mRNA and a powerful new approach for future investigations of translation regulation.

The translation of mRNA by the ribosome requires the precise orchestration of factor and tRNA selection, energy expenditure and step-wise, directional motions through potentially structured templates. Mechanistic complexity is also contributed by the ribosome itself, an enzyme composed of more than 60 individual gene products. The factors required for basal translation also continue to be augmented by an ever-expanding repertoire of cellular components and small molecules providing unique regulatory control mechanisms^{1,2}. Not surprisingly, therefore, efforts to reconstitute translation reactions in combination with direct imaging methods have faced significant challenges. A recent report by Wen *et al.*³ marks a triumphant stride in biophysical investigations of protein synthesis, offering a powerful new avenue for exploring the molecular basis of directional ribosome motion at the single-molecule scale. Their success, founded on a confluence of experience and knowledge spanning biology, chemistry, physics and years of collaborative efforts, is a tour de force in both the single-molecule and translation fields. The result is an unveiling of the first approach to “seeing” step-wise progressions of the ribosome through structured mRNA templates.

Single-molecule studies offer a powerful means to investigate complex biological systems. Observing a multistep process one molecule at a time dramatically aids in delineating the order and timing of discrete events obscured in bulk investigations. Instruments capable of measuring ribosome functions at nanometer resolution have been developed only recently^{4–7}. Wen *et al.*³ now present observations of processive ribosome movements along mRNA and a wealth of insights about the complex reaction coordinate underpinning directional translation. They began by coupling the 5′ and 3′ termini of mRNA to silica beads. This allowed them to trap individual mRNAs using micropipets and/or focused beams of infrared light, and to extend them by force. By carefully designing mRNA secondary structures and controlling the pulling force, the ribosome’s step-wise translocation events could be monitored (Fig. 1).

The authors first demonstrated that the ribosome moves in discrete, “comma-free” codon steps. This finding, portended by a long history of biochemical and biophysical investigations⁸, provides reassurance that their system recapitulates biological reality. Next, with the benefit of single-molecule observations, the authors were able to dig deeper into the underlying mechanism of ribosome movement. First, they address the observation that the ribosome’s step-wise transit along mRNA is punctuated by pauses of broadly distributed duration. While these pauses are minimally composed of the times for the biochemical reactions of tRNA selection, peptide bond formation and elongation factor G binding, nontrivial amounts of time may also be required for conformational changes within the

ribosome that are likely to precede translocation^{9,10}. Shine-Dalgarno– like base pairing interactions between rRNA and mRNA, much like those that take place during bacterial translation initiation¹¹, contributed to the longest pauses (up to 1–2 min). The mechanistic inference is that mRNA-ribosome interactions may become rate limiting in global protein synthesis, which provides a regulation point for noncanonical translation events such as recoding and/or frameshifting¹². These insights were amplified by a careful analysis of shorter pauses (about 1–3 s) specifically observed in runs of “active” translation. The distribution of pause durations was not consistent with a single rate-determining barrier, but instead a convolution of two rate-determining steps. One of these steps was shown to be strongly force dependent: pause durations decreased by imparting tension on the trapped mRNA, and paused complexes could be rescued by applying force.

Measurements of the velocity of ribosome movement offered additional clues. The distribution of translocation times could be well described only by three rate-determining processes. Each step contributed equally, occurring over approximately 25 ms (~75 ms total). Interestingly, translocation times were not force dependent. Thus, the authors concluded that it is the formation of an intermediate state before ribosome stepping and mRNA unwinding that is force dependent. Though the application of force presumably shortens the waiting time for the mRNA unfolding transition, global conformational changes within the ribosome, as well as the formation of tRNA hybrid states, may also contribute sizable activation barriers to movement that could be affected by mRNA tension. Future investigations will be required to delineate the precise nature of this force-dependent, rate-limiting step preceding translocation. Here, investigations of the ribosome’s intrinsic helicase activity¹³, as well as the conformational events taking place within the ribosome before translocation, may help delineate the observed barriers to motion. As noted in the paper, the three rate-determining steps underpinning the ribosome’s movement by one codon may correspond to its movement by three nucleotides. It will also be important to test this intriguing hypothesis through pinpointed experiments testing the sequence dependence of translocation times and/or the time observed for frameshifting events where two or four nucleotides are transited. Elaborations on this new system may also provide a powerful tool for revealing the impact of mRNA binding proteins and small RNAs on forward progression as well as factors that promote reverse translocation¹⁴.

A continued evolution of equipment and biochemical technologies is certain to afford improvements in signal-to-noise ratios, time resolution and new investigations beyond this already lofty endeavor. Enhanced perspectives into the nature of the translation mechanism and its regulation are sure to ensue. Collaborative groups may be ideally positioned for next-generation experiments using integrated single-molecule fluorescence and optical trapping instruments. Such systems may be used to simultaneously address the precise contributions of biochemical and conformational processes underpinning translational pausing and directional ribosome motion. Such pioneering research, along with continued developments in molecular dynamics simulations of the translation machinery¹⁵, will encounter significant challenges and risks in the short term. However, it is only through continued investments of this kind that we can hope to attain deeper knowledge of the physical mechanism driving this vital and complex biological process.

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Figure 1. Stepping of the ribosome through structured mRNA templates observed by single-molecule optical trapping methods. The basic design of the optical trap used to monitor translation (top). Actual data demonstrating that ribosome activity unzips mRNA secondary structure, which results in measurable extensions of the trapped mRNA (bottom).