Tracking transcription-translation coupling in real-time

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A central question in biology is how macromolecular machines function cooperatively. In bacteria, transcription and translation occur in the same cellular compartment, and can be physically and functionally coupled. While several recently published high-resolution structures of the ribosome-RNA polymerase (RNAP) complex provided first mechanistic insights into the coupling process, we lack knowledge of how these structural snapshots are placed along a dynamic reaction trajectory. Here, we reconstitute a complete and active transcription-translation system and develop multi-color single-molecule fluorescence microscopy experiments to directly and simultaneously track transcription elongation, translation elongation and the physical and functional coupling between the ribosome and the RNAP in real-time (Qureshi & Duss, bioRxiv, 2023, 10.1101/2023.12.07.570708). Our data show that physical coupling between ribosome and RNAP can occur over hundreds of nucleotides of intervening mRNA, by mRNA looping, a process facilitated by transcription factor NusG. We detect active transcription elongation during mRNA looping and show that transcription factor NusA-paused RNAPs can be activated by the ribosome by long-range physical coupling. On the other hand, the ribosome slows down while colliding with the RNAP, a state with no intervening mRNA between both machines and physical coupling between both machineries becomes more transient once the RNAP escapes from a collision. We hereby provide an alternative explanation on how the ribosome can efficiently rescue RNAP from frequent pausing without requiring collisions by a closely trailing ribosome. Overall, our dynamic data mechanistically highlight an example of how two central macromolecular machines, the ribosome and RNAP, can physically and functionally cooperate to optimize gene expression.