STRUCTURAL BIOLOGY

Ribosomes, start your engines

Tracking single molecules in real time reveals the hidden dynamics of translational initiation.

The cartoons in molecular biology textbooks give the comforting impression that we know all about the events that kick-start the ribosome to begin translating protein. Although it is true that the global mechanism and rate of initiation are known, the exact sequence and timing of factors that enter and leave the initiation complex—the compositional dynamics—are still mysteries that keep researchers like Joseph Puglisi of Stanford University in hot pursuit.

Standard biophysical experiments tend to average out information on timing and variability in molecular behavior. Single-molecule methods can get around the problem, but they do not mimic conditions *in vivo*. "We were always limited in the concentration of fluorescently labeled components that we could throw in and observe [because of high background]," says Puglisi. This led Puglisi and his group to adapt the Pacific Biosciences single-molecule sequencing platform to image translation at component concentrations close to what is seen in the cell. They previously had published work detailing the transit of transfer RNA in single elongating ribosomes and now describe the dynamics of the regulated and rate-limiting initiation process.

Their approach relies on zero-mode waveguides, nanostructures that create an optical trick whereby lasers illuminate only a tiny volume near a glass viewing window. Single messenger RNA molecules tethered to the glass have access to high concentrations of dye-labeled protein, transfer RNA or ribosomes, but the system detects only bound components. They observed up to four components simultaneously and in real time using different color fluorophores.

The group found that initiation does not proceed in a rigid sequence. Multiple pathways are sampled, and the order of arrival on the messenger RNA depends on the local concentration of components. "We're trained to think in terms of linear, simple chemistry behavior," says Puglisi, "but that's often not how these processes occur."

The hardest part of the approach is labeling proteins without affecting their function, which takes careful control experiments and is especially challenging for complexes with many components.

Puglisi is now studying other less-understood initiation factors and eukaryotic initiation. "I hope people grab onto this as a more general way to look at complex biology, as another tool in the arsenal," he says.

Tal Nawy

RESEARCH PAPERS

Tsai A. *et al*. Heterogeneous pathways and timing of factor departure during translation initiation. *Nature* advance online publication (17 June 2012).

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