IMAGING

RNA catch and release

The binding of single RNA molecules to individual proteins can be observed in the subcellular compartments of living cells.

The localization and translational regulation of mRNAs within the cell rely on interactions with a host of protein factors. While we know that interactions with proteins spatiotemporally regulate mRNA activity, to date it had not been possible to observe these interactions as they occur within a cell at the single-molecule level.

A group led by Robert Singer and Bin Wu at the Albert Einstein College of Medicine now tracks, at the single-molecule level in live cells, the interactions of a model mRNA with the RNA-binding protein ZBP1 and ribosomes.

Singer had previously hypothesized—in a paper published 10 years ago—that the protein ZBP1 binds certain mRNAs to restrict their translation until they reach the cell's leading edge. This presumably occurs when ZBP1 is phosphorylated by a membrane-localized kinase, releasing the mRNA locally to allow ribosome binding and translation. However, "We didn't have a way of [directly] testing this hypothesis [*in vivo*]," Singer recalls.

To accomplish this, Singer, Wu and their colleagues have taken advantage of recent developments in fluorescence correlation spectroscopy (FCS). The approach uses two-photon microscopy to excite two fluorescent probes in the same tiny volume. "The beauty of the technique is that the way FCS works, molecules passing through the excited spot give you photons, and if you have two molecules of different colors bound to one another, the temporal correlation of those photons will be superimposed—telling you that the two molecules are traveling together in a complex," explains Singer, who also points out that the technology itself is not new. "FCS has been around since the 1970s, but I think what this [application] demonstrates [is that] it can be used to get to this particular type of biological information," he says, referring to single molecule–level interaction between proteins and RNAs.

By calculating the total amount of correlated brightness, an approach known as fluorescence fluctuation spectroscopy (FFS), it is also possible to determine how many protein molecules are bound to a single labeled RNA. This can be done in precise cellular locations, given the capacity for a femtoliter-sized observation volume.

Showing that the method could achieve the necessary resolution was not trivial, however. "One of the things that held up the technological application in this case is that we have to detect a single protein on a single RNA. To show that this technique was capable of single-protein detection was the critical step," Singer notes.

To directly test the hypothesis that ZBP1 prevents the β-actin mRNA from being translated until it reaches the cell's outer edge, Singer's team used their method on cells expressing ZBP1 and β-actin mRNA—both fluorescently labeled—at levels similar to those of the endogenous species. Wild-type ZBP1 was found to bind β-actin mRNA predominantly at the nuclear periphery, whereas a mutant ZBP1 that cannot become phosphorylated showed increased interaction at the cell's leading edge. Conversely, using mCherry-tagged ribosomal protein L10A as a probe, they observed greater interaction with the β-actin mRNA at the cell's leading edge, and this preferential distribution was lost in ZBP1-null cells. These observations are in perfect agreement with the model Singer proposed a decade ago, also confirming the power of the approach.

Although this new method requires a specific setup and is not yet routine, it is in principle achievable with any existing two-photon microscope capable of photon counting. "If the experiments can be done, the equipment will follow as people want to use it, and acceptance of this technique will increase," Singer predicts.

As this implementation of FFS is adopted, it may allow several central questions in cell biology to be answered. "Can we now develop that technique to allow seeing the translation of single RNA molecules, when they translate, how long they translate? That is the next step in our view," says Singer.

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RESEARCH PAPERS

Wu, B. *et al.* Quantifying protein-mRNA interactions in single live cells. *Cell* **162**, 211–220 (2015).