MOLECULAR BIOLOGY

Watching translation of single mRNAs in cells

Four studies describe methods to image the dynamics of translation at the single-transcript level in real time in living cells, with exciting results.

Protein synthesis is a fundamental biological process, and proper regulation of translation is crucial for cell viability. Although major methodological advances such as ribosome profiling have generated important insights into translation at the genome level, studying translation at the single-mRNA level in living cells has proven challenging.

Four independent studies recently tackled this issue, reporting strikingly similar methods for studying translation at the single-molecule level in living cells. The basic principle of all four experimental setups is that an mRNA transcript is engineered to encode a series of epitopes that recruit fluorescently labeled binding proteins to the site of nascent synthesis. The transcripts themselves are made fluorescent by labeling of the 3′ UTR, which allows the mRNAs to be imaged independent of active translation.

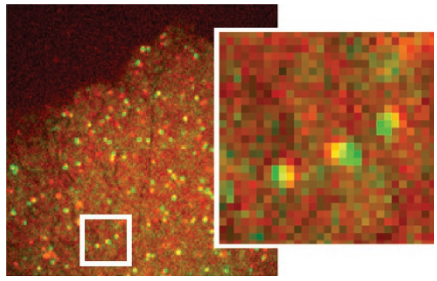
Studies by the groups of Marvin Tanenbaum at the Hubrecht Institute, Robert Singer at Albert Einstein College of Medicine and Xiaowei Zhuang at Harvard University made use of SunTags for labeling the protein during synthesis. The SunTag system is composed of a transcript that expresses the epitope sequence and a synthetic single-chain intracellular antibody (scFv) fused to GFP that binds the epitope. For mRNA labeling, binding sites for PP7 or MS2 were engineered into the 3′ UTR of the transcript, allowing labeling by the RNA-binding proteins PP7 or MS2 fused to a fluorescent tag.

The approach used by Timothy Stasevich at Colorado State University and colleagues was similar, but rather than expressing SunTag, they expressed 'spaghetti monster', composed of ten FLAG-tag sequences in a row upstream of the protein-coding region. This was bound by fragments of anti-FLAG that were labeled with Cy3. Their mRNA was labeled analogously to that used in the other studies. Both strategies allowed the position of individual mRNAs and translation of the mRNAs to be monitored simultaneously.

Importantly, these methods allowed the researchers to gain quantitative insight into translation as it occurs in cells. For example, Stasevich and colleagues looked at transcripts encoding three different genes and found that they were all translated in polysomes, in which a single mRNA is translated by multiple ribosomes. The number of ribosomes per polysome differed among transcripts, ranging between two and five. The team was also able to use fluctuation correlation spectroscopy to study translation kinetics. For one transcript, they observed a rate of approximately ten amino acids per second. The team also used a second spaghetti monster composed of ten HA tags to image two transcripts being translated in the same cell. Using this approach, they found that most polysomes acted independently, but a small fraction of the polysomes synthesized both HA- and FLAG-tagged proteins, indicating that two polysomes for different mRNAs can occasionally form higher-order complexes.

Zhuang and colleagues demonstrated that their method can monitor transient translational regulation. They examined stressed cells and observed pulse-like translation of transcripts containing the upstream open reading frames of a gene known to be upregulated during unfolded protein stress. They were also able to observe local translation in neuronal dendrites, and they demonstrated that mRNAs being translated in polysomes can undergo active transport.

Singer and his team studied translation in a variety of settings. In one case, they imaged translation at the endoplasmic reticulum (ER) by fusing their epitope and tag-bearing mRNA with an N-terminal ER targeting signal. Those studies allowed them to observe cotranslational insertion of the proteins into the ER and show that the mRNAs are tethered to the ER only during active translation. Singer's group also investigated local translation in neurons and found that mRNAs can undergo translation during transport.



Imaging transcript position (red) and active translation (green) in live mammalian cells. The right-hand panel shows a magnified view of the region enclosed by the white box in the left-hand panel. Figure adapted from Yan *et al.* (2016) with permission from Elsevier.

In addition to addressing similar questions as the other groups, Tanenbaum and colleagues used their method to image ribosome stalling. The researchers looked for colocalized protein and mRNA signals after the addition of translation-initiation inhibitors. They observed stalling for 5–10% of the labeled mRNAs. Further analysis showed that stalling is probably caused by damaged mRNA and that stalling is not always rapidly targeted by no-go mRNA decay machinery. In separate experiments, the team observed that mRNAs of a given type are not translated with equal efficiency, even in the same cell.

These studies highlight the power of these approaches for studying translation in real time at the single-mRNA level in living cells, and they reveal the remarkable heterogeneity of translation and the precise nature of its regulation.

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RESEARCH PAPERS

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