SENSORS AND PROBES

Hijacking Cas9 for live-cell RNA imaging

Researchers target fluorescent protein–Cas9 fusions to specific mRNAs for tracking in live cells.

RNAs are a vitally important class of biomolecules that function at every level of gene expression. However, tools for imaging RNA in living cells lag behind analogous methods for imaging protein abundance, localization and dynamics. The classic approach for imaging RNA abundance and localization in cells is fluorescence *in situ* hybridization (FISH). Although powerful, FISH requires cell fixation, which is not compatible with live-cell imaging. However, most tools for live-cell RNA imaging involve genetically tagging the RNA of interest.

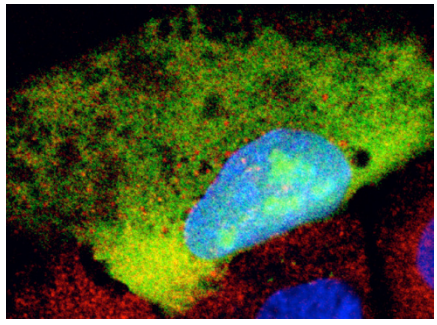
Gene Yeo and his graduate student David Nelles at the University of California, San Diego, sought to overcome these limitations using CRISPR/Cas9 technology, which has become wildly popular for its use in targeted genome editing. Commonly used CRISPR/Cas9 systems are composed of two key elements: the Cas9 protein and a single guide RNA (sgRNA). Cas9 binds to the sgRNA, and the complex then targets a site of interest in the genome determined by complementarity to that site in the sgRNA.

In most circumstances, the CRISPR/Cas9 system is used to target DNA. However, Yeo recalls, "my lab came to the quick realization that the Cas9–sgRNA complex may be hijacked for RNA recognition." He also notes that by the time they had begun their work, "our friend and colleague Jennifer Doudna showed that the complex could recognize RNA *in vitro*." So they began collaborating with Doudna and Mitchell O'Connell, a postdoc in her laboratory, to develop the system further for recognition of RNA in live cells.

Their method involves targeting Cas9 to RNA rather than DNA. Under normal circumstances, Cas9 is specific for DNA because it requires a protospacer-adjacent motif (PAM) that resides next to the target site, but on the opposite strand, for binding. Instead, Yeo's team introduced the PAM *in trans* to the target single-stranded RNA using a DNA oligonucleotide (known as a PAMmer). The team also had to use a mutant of Cas9 that is enzymatically inactive and a chemically modified PAMmer to avoid RNA cleavage. Finally, they tagged Cas9 with a fluorescent protein to light up the RNAs of interest for imaging.

In their first set of experiments, the researchers demonstrated that Cas9–mCherry could specifically recognize an mRNA in the cell's nucleus and then be properly exported into the cytoplasm along with the RNA. These experiments also revealed that the sgRNA was the key player in RNA targeting, but that the PAMmer also contributed to binding efficiency. The team then demonstrated that targeting is specific by showing colocalization with FISH against the same mRNAs in cells. They note that these experiments in particular were technically challenging because they used different modalities of RNA recognition and signal amplification. They also found the results to be better than expected. "Dave and I were surprised that a single dCas9–GFP was able to correlate reasonably with RNA FISH measurements which typically involve tens to dozens (sometimes up to 50) of probes," explains Yeo.

The researchers also demonstrated the power of this method for live-cell imaging by monitoring mRNA trafficking into stress granules in cells undergoing oxidative stress. In those experiments they individually monitored three different mRNA molecules during stress using Cas9–GFP in cells that also expressed a marker for stress granules. In all cases, the mRNAs, which were initially distributed in the cytoplasm, became localized over time in the stress granules.



Cellular RNA imaged with FISH (red) and RCas9–GFP (green). Blue signal represents nuclear DNA. Adapted from Nelles *et al.* (2016) with permission from Elsevier.

The team also tested whether imaging with RNA-targeting Cas9 (RCas9) affects the stability of the targeted mRNA or the expression of the encoded protein. In investigations of both questions, they saw no change in mRNA or protein levels upon targeting, indicating that these factors are not perturbed by this labeling strategy.

The potential applications of RCas9 are seemingly boundless. Yeo says that his team is working hard to improve the sensitivity and resolution of the technology for RNA localization, and they are also interested in identifying other Cas proteins that can be used for RNA recognition. Such proteins could be useful tools for multiplexed live-cell RNA imaging. In addition, outside of imaging, RCas9 could be used to site-specifically target proteins of interest to RNAs to allow scientists to study their functions. This work should also move Yeo and his team closer to other research goals, such as understanding how RNA localization and transport are involved in proper health and in neurological diseases such as amyotrophic lateral sclerosis.

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RESEARCH PAPERS

Nelles, D.A. *et al.* Programmable RNA tracking in live cells with CRISPR/Cas9. *Cell* **165**, 1–9 (2016).