An RNA TREAT for Halloween

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An RNA TREAT. Measuring RNA degradation in single cells. Credit: Friedrich Miescher Institute for Biomedical Research

Jeff Chao, Junior group leader at the FMI, and his group developed a sophisticated method to measure mRNA degradation in single cells. They developed a fluorescent biosensor that allows the distinction of intact transcripts and degradation intermediates. This novel method, known as TREAT, nicely complements the method they developed earlier, called TRICK, that measures protein translation.

Life of an RNA starts in the nucleus, where it is synthesized and further modified –capped, spliced and polyadenylated. It then moves into the cytoplasm where it is translated into a protein, and eventually degraded. The amount of protein produced depends largely on the regulation of transcription, but also the abundance of mRNA. As FMI Group leader,

Jeff Chao points out, "It has become increasingly clear that the regulation of mRNA degradation, particularly during development or rapid environmental changes, can dramatically influence RNA levels." While many of the RNA degradation steps have been characterized, a clear understanding of when and where degradation happens has been missing so far.

Jeff Chao and his team have now developed a fluorescent microscopy method that allows them to measure the spatial and the temporal dynamics of the degradation of single mRNA molecules in living cells.

Chao and his colleagues took advantage of a viral RNA structure that forms a knot-like structure. This pseudo-knot prevents the degradation of mRNA by Xrn1, a 5'-3' exoribonuclease. Chao explains: "With the help of a multicolored biosensor containing these viral pseudo-knots, we were able to distinguish between intact mRNA transcripts and mRNA transcripts that are being degraded." The scientists called this technique TREAT for 3(Three)'-RNA End Accumulation during Turnover.

First and most importantly, TREAT allowed the scientist to observe mRNA degradation in real-time in living cells. To visualize degradation, the scientists engineered a transcript that is labeled with two RNA-binding proteins fused to two distinct fluorescent tags: one of the proteins – PP7 (tagged with green fluorescent protein) – binds to the coding region of the mRNA, while the other – MS2 (with a red tag) – binds to the 3' untranslated region. Between PP7 and MS2, the scientists introduced the viral pseudo-knots. Thus labeled, the individual untranslated mRNAs appear yellow. As the RNA is degraded by XRN1, the green-tagged PP7 is displaced. However, at the position of the pseudo-knot, XRN1 degradation halts, which allows the detection of a quantifiable color change from yellow to red.

In addition, Chao comments: "Using TREAT, we have been able to

measure mRNA decay in single cells and found that individual degradation events occur independently in the cytosol. The degraded mRNAs did not accumulate in processing bodies." These are important first insights because the processing bodies were thought to play a direct role in RNA degradation. Processing bodies are membrane-less compartments that form during phase transitions. They are comprised of RNA-binding proteins and mRNAs, and since they contain many proteins involved in mRNA turnover, it was proposed that they are cellular sites of RNA degradation.

"TREAT nicely complements the other technology that we developed earlier called TRICK. With the selection of appropriate fluorescent markers, we can now monitor both RNA turnover and RNA translation 'live', and we can address how these processes are interconnected within a single cell," says Chao.

More information: Ivana Horvathova et al. The Dynamics of mRNA Turnover Revealed by Single-Molecule Imaging in Single Cells, *Molecular Cell* (2017). DOI: 10.1016/j.molcel.2017.09.030

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