Nature Reviews Genetics | Published online 30 Mar 2016; doi:10.1038/nrg.2016.41

TECHNIQUE

Transcript tracking by CRISPR

RNA transcription, processing and trafficking are key steps in gene expression, hence tracking particular RNAs in living cells can provide valuable biological insights. A new study adapts the CRISPR–Cas9 genome-editing system to act as a programmable real-time transcript probe.

The most common method for characterizing transcript levels and localization in cells is fluorescence *in situ* hybridization (FISH), which is based on the hybridization of fluorescent probes to target transcripts. However, the requirement for cell fixation in FISH prevents temporal analyses of the same cells and has motivated the development of transcript tracking methods in live cells. One major challenge for live-cell transcript tracking has been to develop a system that can be readily programmed for any chosen target transcript, without the need to incorporate engineered protein-binding sites.

To create a programmable transcript probe, Nelles *et al*. took advantage of different modified CRISPR–Cas components. First, a nuclease-deficient Cas9 protein was fused to nuclear localization sequences and a red or green fluorescent protein. This strategy allows the Cas9 fusion protein to act as a fluorescence-based molecular probe that will not cleave its nucleic-acid target and will remain restricted to the nucleus unless bound to an exported nucleic acid. Next, two nucleic acid components were designed to hybridize nearby on a transcript of interest: a guide RNA (gRNA) and a proximal adjacent motif (PAM)-containing mixed DNA–RNA molecule (PAMmer). The aim of the gRNA and PAMmer combination is to recruit the Cas9 fusion to copies of the target transcripts; they are designed to target regions without endogenous PAM sequences to avoid binding to the corresponding genomic locus.

To test the system, the investigators transfected expression vectors for the Cas9 fusion and gRNA, as well as *in vitro* synthesized PAMmer, into human cell lines. A non-targeting gRNA–PAMmer pair resulted in Cas9 retention in the nucleus, whereas targeting the gRNA and PAMmer to the *GAPDH* transcript resulted in >80% of Cas9 protein being exported to the cytoplasm.

Additional testing of the method by targeting different transcripts, including *ACTB*, *CCNA2* and *TFRC*, revealed that this system does not overtly perturb the endogenous expression of the transcripts or the encoded proteins, that characterization of transcript localization in live cells using a single gRNA–PAMmer pair performs comparably to FISH using tens of probes in fixed cells, and that transcript relocalization in response to oxidative stress can be tracked in real time.

Such an approach has the potential to be applied to diverse transcript types in multiple cell systems, including the differential detection of splicing isoforms using gRNA–PAMmer pairs across exon junctions. It will be interesting to see whether the use of a synthetic transfected PAMmer can be overcome: if all components can be DNA encoded, additional long-term and *in vivo* possibilities may arise. Beyond detecting RNA, further adaptations could enable the site-specific manipulation of target RNAs, such as altering RNA modifications.

Darren J. Burgess

ORIGINAL ARTICLE Nelles, D. A. *et al.* Programmable RNA tracking in live cells with CRISPR/Cas9. *Cell* http://dx.doi.org/10.1016/j.cell.2016.02.054 (2016)
FURTHER READING Crosetto, N., Bienko, M. & van Oudenaarden, A. Spatially resolved transcriptomics and beyond. *Nat. Rev. Genet.* **16**, 57–66 (2015)