GENOMICS

RNA detection with C2c2

The discovery of two distinct endonuclease activities in the C2c2 protein explains how template RNAs are processed in type VI CRISPR systems and enables the development of a sensitive RNA detection system.

In the bacterial adaptive immune system, clustered, regularly interspaced short palindromic repeats (CRISPRs) direct cleavage of target nucleic acids. Although most CRISPR systems target DNA, the type VI system with its C2c2 endonuclease uses RNA guides to target single-stranded RNA (ssRNA). However, type VI systems have no obvious means to process precursor CRISPR RNA (crRNA) into mature crRNAs that can be used as templates to degrade target ssRNA. This led Jennifer Doudna of the University of California, Berkeley, and Lawrence Berkeley National Laboratory and colleagues to investigate whether C2c2 itself could process precursor crRNA and whether the properties of C2c2 could be adapted to develop a method for the detection of specific RNAs.

In vitro assays with purified C2c2 proteins and precursor crRNAs demonstrated that C2c2 was indeed able to produce mature crRNAs through an endonuclease activity distinct from that used to degrade its RNA targets. This activity was dependent on both the structure and the sequence of the template, which explained how C2c2 prevents the loading of RNAs that do not originate from type VI CRISPR loci.

Once C2c2 detects a target ssRNA complementary to the crRNA template, nonspecific endonuclease activity is activated and nontarget RNA is degraded *in trans*. This prompted the authors to test whether the template surveillance system of C2c2 could be harnessed to detect specific RNAs within a pool of transcripts. The researchers designed a fluorophore-quencher-labeled RNA that would be degraded once C2c2 was activated, thereby releasing the fluorophore. Degradation of this nontarget transcript would therefore lead to fluorescence when C2c2 detected a specific target RNA complementary to the crRNA. To test the system, they introduced the reporter RNA, in combination with C2c2 loaded with bacteriophage-λ-targeting template crRNAs, into HeLa cells. Fluorescence could be detected when as little as 10 picomolar foreign λ target RNA was spiked into the cells. The system could also detect endogenous β-actin mRNA from a pool of human, but not *Escherichia coli*, RNA, demonstrating specificity.

Transcript detection using C2c2 is both more direct than polymerase-based methods and readily adaptable to specific targets, and thus offers a new tool for RNA detection.

Richard Pattison

RESEARCH PAPERS

East-Seletsky, A. *et al.* Two distinct RNase activities of CRISPR-C2c2 enable guide-RNA processing and RNA detection. *Nature* **538**, 270–273 (2016).