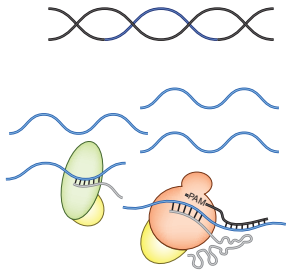
CRISPR targets RNA

Having revolutionized DNA editing, CRISPR turns to RNA.

Adapting the CRISPR bacterial immune system to eukaryotic genomes has enabled an unprecedented ability to modify DNA from disabling to swapping or tagging genes, to introducing specific point mutations, to enhancing or repressing select genes' activities and carrying out genome-wide functional screens. In the CRISPR system, a short guide RNA (gRNA) molecule steers an endonuclease to a target sequence complementary to the gRNA and located next to a protospacer-adjacent motif (PAM).

While CRISPR has mostly been applied to DNA, recent advances have expanded its range to RNA editing. RNA interference has been a mainstay to knockdown gene expression, but to target particular transcripts for imaging or subcellular localization has required cumbersome design of RNA aptamers or proteins such as Pumilio for each target RNA.

To target RNA in a way that can easily be programmed and scaled up, two independent



C2c2 (green) or dCas9 with a PAM-providing DNA oligo (orange) and their respective guide RNAs (gray) bring effector proteins (yellow) to RNA (blue).

groups took different routes. Gene Yeo, in collaboration with Jennifer Doudna, expanded on previous findings from the Doudna lab showing that Cas9 can be targeted to RNA if the PAM is provided by a separate DNA oligonucleotide that binds the target RNA. The researchers recently demonstrated that this approach can target specific RNAs; a fusion between a catalytically inactive Cas9 and GFP allowed them to track the subcellular localization of RNA in live mammalian cells (*Cell* **165**, 488–496, 2016). The teams of Feng Zhang and Eugene Koonin took a different approach. Instead of Cas9, they used the C2c2 nuclease, which has an RNase domain but no known DNase domains. They targeted C2c2 by a single 28-nucleotide gRNA and saw single-strand RNA cleavage in the bacterial target transcripts (*Science* **353**, aaf5573, 2016). Simple base substitutions converted C2c2 into a catalytically inactive RNA binding protein that can now be coupled to different effector proteins.

Both approaches open the possibility of influencing the many post-transcriptional processing steps of RNA. C2c2 is a particularly good candidate, since recent work on its catalytic activity showed that it has two independent RNase activities, one to process its own RNA guides and another to cleave the target RNA (*Nature* **538**, 270–273, 2016), which will allow multiplexed applications.

With the appropriate effector fused to the nuclease, one can envision a myriad of uses, such as regulating splicing, directing RNAs to particular subcellular localizations, attaching or removing chemical modifications, and affecting degradation, to name only a few. RNA-targeted CRISPR will give researchers access to a regulatory layer of which we have so far only scratched the surface. **Nicole Rusk**

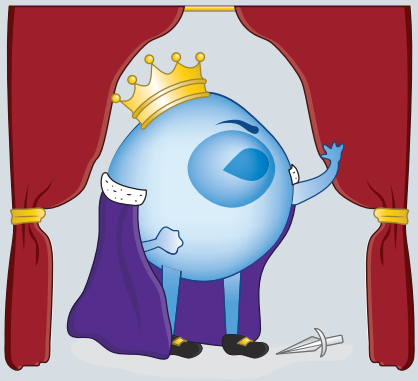
How single cells do it

Single-cell sequencing is poised to elucidate how cells contribute to tissue function.

Single-cell sequencing has cracked open the problem of tissue heterogeneity and enabled the study of new cell types and rare cell populations. Novel applications and analytical tools are now putting emphasis on inferring the functional roles of cells in tissues and developmental events, as well as the genetic programs that drive them.

Transcriptional similarity is widely used to categorize individual cells within a tissue. The same data can also provide functional insight into cell states. Researchers are increasingly using single-cell data to identify cell-type-specific markers and then label and map these cell types back in the intact tissue. For example, one study identified many rare cell types in the gut that likely function in secretion (*Nature* **525**, 251–255, 2015), while another combined unbiased single-cell RNA-seq with imaging of the mouse sympathetic ganglion to reveal specific neuronal populations that innervate the muscles behind goosebumps and nipple erection (*Nat. Neurosci.* **19**, 1331–1340, 2016).

Computational approaches are also being developed to infer the gene-regulatory changes that drive differences in cell state. Pseudotime inference can place single cells along reconstructed developmental trajectories, making it possible to pick out the cells involved in developmental transitions. Better methods are needed to extract the gene regulatory changes that drive these transitions and cellular decisions. To understand transitions in the blood cell differentiation cascade, one approach profiled single-cell gene expression from mixtures of blood cells at different states and generated a dynamic model of the underlying transcription factor regulatory networks (*Nat. Biotechnol.* **33**, 269–276, 2015). Time-course experiments will be more feasible as single-cell sequencing becomes less expensive and more accessible, and the added dimension should help researchers to glean which changes are causal with respect to cell state.



Marina Corral Spence/Springer Nature

Single-cell sequencing helps define a cell's role and how it is played.

Perturbation experiments are also promising routes to finding function. Combining the CRISPR editing system with single-cell studies will be a powerful way to screen for the effect of gene knockouts on single-cell transcription and cellular phenotypes. Now that single-cell RNA sequencing has become routine at large scales, we look forward to experimental and analytical developments that shed light on cellular functions. **Tal Nawy**