Co-opting CRISPR to deliver functional RNAs

Pei-Chun Lin & Jacob E Corn

Portions of the CRISPR-Cas9 guide RNA can be reworked to carry functional RNA cargos to genomic loci.

Long noncoding RNAs (lncRNAs) affect many fundamental biological processes, from transcription and RNA splicing to translation, and the study of their function has given rise to an exciting and rapidly expanding field. However, it can be tricky to separate the targeting and effector functions of a lncRNA, and modular tools are always welcome. In this issue, Shechner *et al.*[1] describe CRISPR-Display (CRISP-Disp), a new approach that deploys RNA-based synthetic devices and lncRNAs to a genomic locus via Cas9-mediated targeting, as both a flexible tool for practical applications and a way to better understand lncRNA function.

The recent development of the clustered, regularly interspaced, short palindromic repeats (CRISPR)-Cas9 system has opened the door to precision gene editing in a variety of contexts. In this system, a single guide RNA (sgRNA), through simple rules of base pairing, directs Cas9 nuclease to a DNA sequence of interest, where the DNA is then cleaved[2]. A nuclease-deficient mutant (dCas9) fused to transcriptional repressors or activators has also been used to control gene expression at targeted loci[3]. But could the sgRNA also be engineered to incorporate functional RNA modules or recruit effectors to a locus?

Two requirements must be satisfied for the sgRNA to carry an RNA device: the device must retain functionality, and the resulting Cas9-sgRNA complex must still be targeted correctly. Modified sgRNAs have already been used with an eye toward Cas9's catalytic activity[4,5]. Shechner *et al.*[1] instead explored topologies for dramatically reengineering the sgRNA to contain an additional RNA cargo (**Fig. 1**). Their main functional test for the cargo was whether it could be used to drive transcriptional activation at a specific DNA address either directly from a dCas9–activator protein fusion or indirectly by recruiting an RNA-binding module fused to the transcriptional activator. This latter "bridged" activation approach has recently been used to enable genome-wide transcriptional activation screening and complex transcriptional reprogramming[6,7], but little is known about the general tolerance of an sgRNA to the insertion of complex sequences.

Shechner *et al.*[1] explored the diversity of RNA devices that could be incorporated into sgRNA, basing their experiments on the observation that an internal stem-loop tolerated insertions well. They found that a wide variety of RNA domains, ranging from 80 to 250 nucleotides, could be displayed as part of the sgRNA without interfering with dCas9-mediated targeting. Most strikingly, even a pool of 1.2 million short insertion variants could be incorporated into the dCas9-sgRNA complex, thus demonstrating CRISP-Disp's potential for screening large numbers of RNA devices and identifying new components of synthetic regulators, reporters and scaffolds. The potential expansion of an sgRNA's scaffolding ability seems remarkably modular and could be flexibly used to recruit many different effectors to a given locus.

Like most other short RNAs, sgRNAs are normally expressed via RNA polymerase III (Pol III) promoters, which limits the length of the incorporated RNA device. Pol II

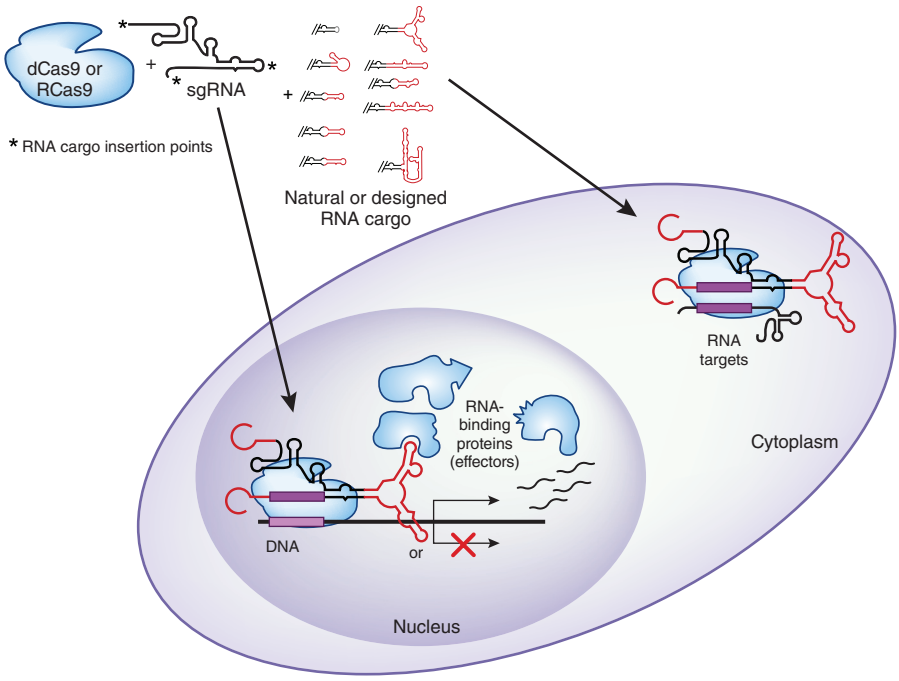


Figure 1 | CRISP-Disp delivers RNA cargos in the cell. A single guide RNA (sgRNA) bound by Cas9 can incorporate a wide variety of RNA devices, delivering them to a specific genomic locus. Cas9 can also be used to specifically target cytoplasmic RNAs (RCas9), suggesting that cargo RNAs could mediate RNA-RNA regulation.

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promoters enable expression of longer transcripts and have recently been used to drive Cas9 multiplexing, such that one long transcript is separated into multiple sgRNAs[8]. Shechner *et al.* instead used Pol II to drive expression of large, natural lncRNA domains of up to 4.8 kilobases incorporated into the ~150-nucleotide sgRNA scaffold[1]. These lncRNAs allowed successful Cas9 targeting, but the incorporated lncRNAs were only modestly functional in terms of their effect on endogenous gene expression at the target site. Future work will no doubt explore whether this reflects context dependence of the lncRNAs under investigation or some other factor.

CRISP-Disp enables a wide range of potential applications, and the authors demonstrate both orthogonal multiplexing of transcriptional activation at multiple loci and live-cell imaging of a genomic locus using a fluorophore-binding RNA aptamer. One can readily imagine CRISP-Disp's use for functional studies of natural lncRNAs and the coupling of multiple artificial noncoding RNA devices for complex transcriptional modulation. The CRISPR-Cas9 system has recently been adapted for RNA targeting[9] (with so-called RCas9), implying that one could use this system to map or alter cellular RNA-RNA interactions (**Fig. 1**). The ability to insert randomized RNA sequences may also enable the *in vitro* selection of new functional RNA modules and the refinement of existing devices. But the limits of RNA sequences and structures that can be successfully incorporated into an sgRNA without disturbing Cas9 targeting have yet to be determined, and the determinants of specificity for even a 'normal' sgRNA are not precisely understood, so one must tread carefully. Regardless, the ability to use Cas9 to deliver an enormous range of RNAs to specific genomic loci opens up many possibilities for the study of natural and artificial lncRNAs.

CRISPR-Cas9 has revolutionized our ability to precisely target nucleic acids in cells and organisms and has provided opportunities to study transcriptional and post-transcriptional regulation. CRISP-Disp is an exciting addition to this toolbox with the potential to generate new insights into the world of noncoding RNAs.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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