Published in final edited form as:

Biochemistry. 2018 January 30; 57(4): 363–364. doi:10.1021/acs.biochem.7b01101.

Programmable RNA binding proteins for imaging and therapeutics

Simone Rauch[1] and **Bryan C. Dickinson**[2,*]

[1]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago IL 60637

[2]Department of Chemistry, University of Chicago, Chicago IL 60637

While RNA was once viewed as a simple intermediate in the Central Dogma, it has now become apparent that RNA provides a critical layer of control to genetic information flow. The lifetime, location, trafficking, and function of RNAs are tightly regulated. Therefore, methods to study the spatial and temporal dynamics of RNAs in physiological conditions are sorely needed. In two recent papers, Abudayyeh *et al.*[1] and Cox *et al.*[2] discovered new Cas family proteins that can be used to deliver protein cargoes to native RNA transcripts in live cells.

The most common method used to obtain spatial information about RNA is fluorescence *in situ* hybridization (FISH). RNA FISH relies on the annealing of an oligonucleotide probe that uses Watson-Crick-Franklin base pair interactions to selectively deliver a fluorophore to the target transcript. Recently, RNA FISH was improved by leveraging Illumina-based "sequencing by synthesis" technology to yield fluorescent *in situ* sequencing (FISSEQ). FISSEQ is based on reverse transcription and cDNA amplification of crosslinked nucleic acids in fixed cells, thereby providing location and abundance information of hundreds of RNA transcripts. However, RNA FISH and FISSEQ can only provide a snapshot of RNA location as they generally require cell fixation.

Acquisition of spatial *and temporal* information of a target RNA in live samples requires the introduction of an exogenous sequence to the RNA of interest. For example, RNA can be fused to the MS2 hairpin, which can then be detected by binding to a GFP-MS2 fusion protein. The development of fluorescent RNA aptamers simplifies these experiments by eliminating the need to express a detector protein fusion. However, these methods require alteration and overexpression of the target RNA, which likely alter the dynamics being measured.

To study native RNA localization in live cells, fluorescently labeled nucleic acids or molecular beacons can be introduced into cells by microinjection. Alternatively, sequence-specific RNA binding proteins, such as Pumillo proteins, can be fused to imaging modalities to track native RNAs. However, the requirement to reprogram the Pumillo binding domains for each individual target transcript limits the practicality of this method. Overall, the field has been limited by a lack of easily programmable RNA binding proteins.

* Dickinson@uchicago.edu; @chembioBryan on Twitter.

The discovery and characterization of the CRISPR system has revolutionized nucleic acid targeting. Aside from its utility as a programmable nuclease, the CRISPR/Cas9 system has been developed into a reliable and versatile tool to deliver protein cargoes site-selectively to the genome. By fusing cargoes to nuclease-inactivated versions of Cas9, a multitude of epigenetic regulatory and fluorescent proteins have been delivered site-specifically. Up until recently, most work has focused on Cas9 systems that target DNA. Only last year, Nelles *et al.* reported that the widely-used spCas9 system can be adapted to target RNA in live cells by supplementing a small piece of DNA that forms a duplex with the target RNA to provide the required PAM binding site.[3] The resulting RNA-targeting Cas9 (RCas9) system can be used to deliver fluorescent proteins to target RNA and allows imaging of endogenous RNA in live cells. In a recent extension of the RCas9 system, Batra *et al.* developed a site-specific RNA endonuclease by fusing RCas9 to the PIN RNA nuclease domain.[4] However, the need for an additional PAMmer DNA presents some potential challenges and limitations of the RCas9 approach. In 2015, Abudayyeh *et al.* reported the discovery and characterization of the first native RNA-targeting single subunit Cas protein, Cas13a (previously C2c2), and showed that it can be programmed to bind a target RNA.[5]

Now, Abudayyeh *et al.* report the successful development of a Cas13a-based programmable RNA-guided RNA-targeting platform for use in live mammalian cells.[1] First, the authors performed a screen of Cas13a orthologues and found that the protein variant from *Leptotrichia wadei* (LwaCas13a) was the most active. The authors showed that endogenous genes in multiple human cell lines and in rice can be knocked down using Cas13a targeting. Knockdown efficiencies were transcript-dependent with maximal knockdown of 85% and 75% for *KRAS* and *PPIB*, respectively. Since LwaCas13a can process its own guiding sequence from a CRISPR repeat array, multiple spacer sequences interspersed with repeat regions can be introduced for multiplexed targeting. To assess the selectivity of the LwaCas13a system, the authors performed transcriptome-wide mRNA sequencing, revealing no measurable off-target knockdown.

Abudayyeh *et al.* further explored whether LwaCas13a could be used as a programmable RNA binding protein for live-cell RNA tracking. The authors developed a catalytically-inactive ‘dead’ LwaCas13a (dLwaCas13a), which maintained robust RNA binding but no longer cleaves the target transcript. To assess whether dLwaCas13a can be used to track RNAs in live cells, Abudayyeh *et al.* fused the protein to GFP and monitored whether the fusion was exported from the nucleus due to mRNA binding. Nuclear export was observed only when the Cas13a fusion was supplied with a target guide(g)RNA binding to a transcript that gets exported from the nucleus. Moreover, the dLwaCasd13a system could be used to visualize stress granules.

In a follow-up study, Cox *et al.* identified a related Cas13b variant that is smaller and works analogously to LwaCas13a.[2] They again showed efficient transcript-specific knockdown and developed an optimized catalytically-inactive Cas13b (dCas13b). To show the versatility of their system, Cox *et al.* fused the known A-to-I editing enzyme ADAR to dCas13b and reported site-specific deamination. Because inosine is processed analogous to guanosine in cells, this approach allows for an exciting new way to correct disease-relevant mutations at the RNA level.

The development of Cas13a and Cas13b variants as versatile and programmable tools to both cleave target RNA and deliver protein cargoes site-selectively in live cells is an important advance for the field. Like all RNA binding protein-based approaches, there is a possibility that the imaging agent will perturb the native state of the target transcript. In the case of Cas13a, this is potentially a substantial concern, as the protein is quite large (~140 kDa). The protein's size also presents other obstacles, such as an inability to be delivered efficiently by adenovirus systems. The Cas13b system is smaller (~110 kDa) and can be packaged for viral delivery, but is still a large protein complex. In future work, the *in cellulo* RNA binding properties of Cas13 proteins need to be carefully assessed in order to understand the capabilities and limitations of each. Overall, the ability to target RNA directly with genetically-encoded tools and without alteration of the target RNA is a milestone for RNA biology. If the previous five years of DNA-targeting Cas9 technologies are predictive, these new RNA-targeting programmable systems will revolutionize our ability to study and perturb RNAs in endogenous environments.

Acknowledgments

Funding:

The Dickinson Lab is supported by the University of Chicago, the National Institute of General Medical Sciences (R35 GM119840), the National Cancer Institute (R21 CA217754), and the National Institute of Mental Health (RF1 MH114102) of the National Institutes of Health, and a Research Fellowship from the Alfred P. Sloan Foundation.

References

1. Abudayyeh OO, Gootenberg JS, Essletzbichler P, Han S, Joung J, Belanto JJ, Verdine V, Cox DBT, Kellner MJ, Regev A, Lander ES, Voytas DF, Ting AY, Zhang F. RNA Targeting with CRISPR-Cas13. Nature. 2017
2. Cox DBT, Gootenberg JS, Abudayyeh OO, Franklin B, Kellner MJ, Joung J, Zhang F. RNA Editing with CRISPR-Cas13. Science. 2017; 550:eaaq0180.
3. Nelles DA, Fang MY, O'Connell MR, Xu JL, Markmiller SJ, Doudna JA, Yeo GW. Programmable RNA Tracking in Live Cells with CRISPR/Cas9. Cell. 2016; 165(2):488–496. [PubMed: 26997482]
4. Batra R, Nelles DA, Pirie E, Blue SM, Marina RJ, Wang H, Chaim IA, Thomas JD, Zhang N, Nguyen V, Aigner S, Markmiller S, Xia G, Corbett KD, Swanson MS, Yeo GW. Elimination of Toxic Microsatellite Repeat Expansion RNA by RNA-Targeting Cas9. Cell. 2017:1–25.
5. Abudayyeh OO, Gootenberg JS, Konermann S, Joung J, Slaymaker IM, Cox DBT, Shmakov S, Makarova KS, Semenova E, Minakhin L, Severinov K, Regev A, Lander ES, Koonin EV, Zhang F. C2c2 Is a Single-Component Programmable RNA-Guided RNA-Targeting CRISPR Effector. Science. 2016; 353(6299):aaf5573–17. [PubMed: 27256883]