Humanizing Transcriptome Engineering

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Transcriptome targeting offers a tunable and reversible approach for cellular engineering. Accordingly, the ability to precisely perturb RNAs has broad implications for research and therapeutics. In this issue, Rauch and colleagues present a new addition to the RNA engineering toolbox that is modular, versatile, and built using human components.

Short oligonucleotides are a simple and effective approach to site-specifically target RNAs, and they have also been used to serve as guides to recruit novel effector biomolecules through coupling to additional RNA domains. Using this strategy, targeted recruitment of either endogenous or exogenous effectors to specific transcripts has been successively achieved (see Figure 1). For example, to induce targeted RNA degradation or cutting, RNA or DNA oligonucleotides have been used to recruit the RNA-induced silencing complex (RISC) or cellular RNases to the target RNA (Yuan et al., 1992). Post-transcriptional modifications or targeted sequence editing of RNA molecules can be achieved by similar approaches, with RNAs instead designed to recruit endogenous effectors like base editing enzymes (Katrekar et al., 2019; Woolf et al., 1995; Karijolich and Yu, 2011; Merkle et al., 2019). Additionally, guide RNAs containing sequences derived from human (Fukuda et al., 2017; Merkle et al., 2019) or viral (Katrekar et al., 2019; Montiel-Gonzalez et al., 2013) RNA secondary structure elements have been used to recruit fusion proteins containing specific human or viral RNA binding domains (e.g., the MS2 tagging system). Alternatively, RNA targeting can also be achieved through direct interaction with an RNA binding protein (RBP) or ribonucleoprotein (RNP), such as programmable RBPs based on the Pumilio homology domains (Adamala et al., 2016) and the recently described CRISPR-Cas13 systems (Cox et al., 2017). In this issue, Rauch and colleagues present CIRTS (CRISPR-Cas Inspired RNA Targeting System), a new addition to the RNA engineering toolbox that is rationally designed, programmable, and built from human parts (Rauch et al., 2019).

Conceptually, CIRTS is a two-component system comprising a guide RNA and a tri-domain protein bearing a single-strand RNA binding region fused to a hairpin RNA binding region, that in turn is fused to an effector domain. The guide RNA has an engineered hairpin that interacts with the hairpin RNA binding domain to drive ribonucleoprotein complex formation, and the single-stranded RNA binding region interacts with the displayed guide RNA to stabilize it. This displayed guide sequence facilitates target RNA binding, thereby bringing the effector domain into proximity to engineer a host of desired RNA modifications. By swapping effector domains, the authors demonstrate the versatility of the platform via applications ranging from RNA degradation, to translational regulation, to base editing. Notably, the modular CIRTS system is less than half the size of the smallest CRISPR-Cas-based transcriptome engineering tools. This small size of the complex makes it readily packable into *in vivo* delivery vehicles such as adeno-associated viruses. In this regard, it is important to note that, unlike DNA editing approaches where hit-and-run strategies suffice, efficacious RNA targeting needs continuous effector expression. This poses the problem of potential immunogenicity issues for *in vivo* targeting applications when using components derived from other species, for example, bacterial-derived CRISPR-Cas effectors. If delivered as an RNP, there is a need for repeat dosing to maintain therapeutic efficacy and thus the possibility for neutralization of non-native systems by circulating antibodies through B cell activation. If delivered via nucleic-acid-based long-term expression constructs, clearance of treated cells by cytotoxic T lymphocytes is a possibility. Thus, the fully human nature of the CIRTS system opens up the potential for utility in therapeutically relevant settings by overcoming some of these challenges.

In this regard, although it's useful to be able to rely solely on human parts, such a targeting platform is also by design not orthogonal to mammalian cellular processes and can thus potentially interact with other proteins or nucleic acids in the cell. For instance, although global off-targeting was not measured for the CIRTS base editing approach, it is well documented that overexpression of adenosine deaminase acting on RNA (ADAR) will result in significant off-targets throughout the transcriptome. Consequently, exploring the two intertwined aspects of activity and specificity more deeply will be critical for this platform going forward. In this regard, for an RNA targeting toolset, use of guide RNAs that can suffice to recruit endogenous effectors presents the most specific and minimally disruptive modality for cellular engineering. Such a strategy enables exquisite specificity, for example as shown recently for RNA base editing where genetically encodable simple long antisense RNAs (Katrekar et al., 2019) or chemically modified short oligonucleotides bearing an ADAR recruiting double-stranded RNA domain (Merkle et al., 2019) can site-specially recruit endogenous ADARs with extremely low off-target rates. However, such endogenous effector recruitment approaches are reliant on the cell's machinery and are thus missing the key effector expression engineering lever to be able to fully regulate on-target activity at every target

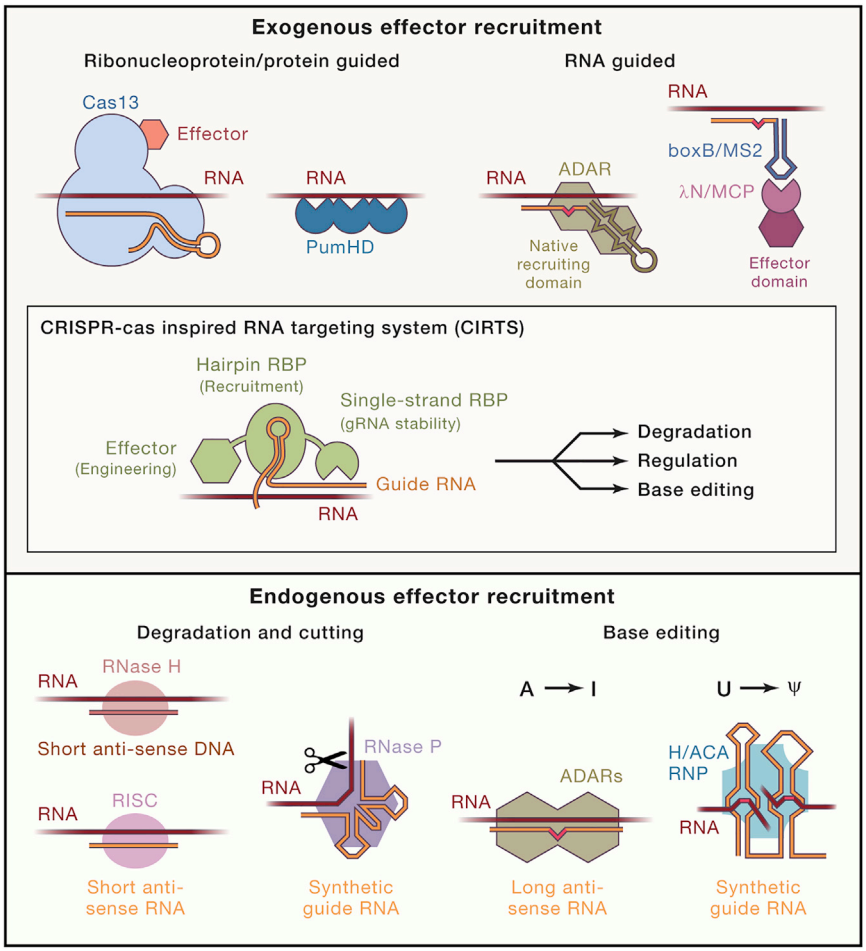

Figure 1. Transcriptome Engineering Strategies

Approaches to target RNA can be broadly classified as those that enable site-specific recruitment of exogenous effectors (delivered into a cell) or site-specific recruitment of endogenous effectors (already present in a cell). Examples of existing methodologies are outlined respectively at the top and bottom. Inspired by the CRISPR-Cas systems, CIRTS (middle) is a new, programmable exogenously delivered platform for versatile transcriptome engineering.

locus and in every desired cell type. Instead, an exogenously delivered system such as CIRTS enables direct control of on-target activity. Correspondingly, the authors demonstrated multiplexability with retained activity for the CIRTS nuclease and CIRTS regulatory modalities. A future broader evaluation across more endogenous targets, and systematic platform improvements through protein and guide engineering, will be important endeavors to boost the current modest activity of CIRTS modules. Also, although presently the single-stranded RNA binding domain serves primarily to stabilize the guide RNA, future avatars might help tune off-targeting rates while maintaining on-target activity. Additionally, as human-based systems run the risk of overwhelming cellular processes by sequestering endogenous interacting components, thus progressively engineering orthogonality to endogenous processes will be another important aspect to explore.

Altogether, CIRTS provides a new chassis for a programmable RNA-guided RNA targeting platform, and with progressive engineering has the potential to evolve into a powerful tool for basic science and therapeutic applications.

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