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Critical review on engineering deaminases for site-directed RNA editing

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The game-changing role of CRISPR/Cas for genome editing draw interest to programmable RNA-guided tools in general. Currently, we see a wave of papers pioneering the CRISPR/Cas system for RNA targeting, and applying them for site-directed RNA editing. Here, we exemplarily compare three recent RNA editing strategies that rely on three distinct RNA targeting mechanisms. We conclude that the CRISPR/Cas system seems not generally superior to other RNA targeting strategies in solving the most pressing problem in the RNA editing field, which is to obtain high efficiency in combination with high specificity. However, once achieved, RNA editing promises to complement or even outcompete DNA editing approaches in therapy, and also in some fields of basic research.

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Introduction

Since the advent of the CRISPR/Cas system [1], a huge effort is made in the engineering of programmable RNA-guided machines. For genome editing, the CRISPR/Cas system was game-changing due to the ability of the large CRISPR/Cas enzymes to melt DNA for enabling the guide RNA (gRNA) to base pair to the target [2]. Compared to preceding approaches, these technologies dramatically simplified the manipulation of genomes. However, it remains to be seen if they are also more suitable for the therapeutic application [3], when safety is more important than ease of use. In this context, the targeting of RNA instead of DNA could be an attractive alternative. When targeting RNA, a nucleotide change will be reversible and tunable in yield. The limited duration will also limit the danger related to harmful off-site editings. Tunability will allow to continuously adjust the (adverse) effect in a dose-dependent manner. Furthermore, many attractive nucleotide changes are inaccessible or ineffective at the genome level, for example, when a gene loss is either lethal or readily compensated [4•,5]. In these terms, the targeting of signaling networks at the RNA level would be particularly attractive [4•,5]. Furthermore, the discovery of new layers of control in the epitranscriptome fortify the need to explore RNA targeting strategies [6,7]. However, we consider it unlikely that the CRISPR/Cas system will be superior to other RNA targeting strategies, as its unique property to melt the DNA, is not required. It is rather that the researcher will have the choice between several strategies, each with its unique benefits. For illustration, we compare three prototypic strategies. The considerations and conclusions should be transferable to many settings.

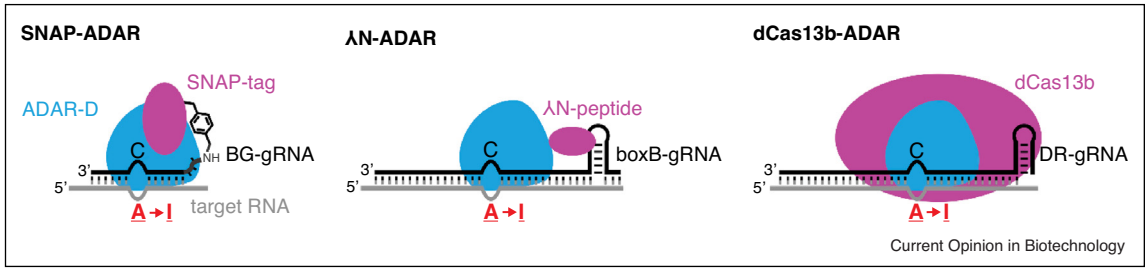
Site-directed A-to-I RNA editing

A-to-I editing refers to the enzymatic hydrolysis of adenosine to inosine in RNA, done by two enzymes, ADAR1 [8] and ADAR2 [9]. As inosine is biochemically read as guanosine, an A-to-G change is formally made into the RNA, which can result in the reprogramming of amino acids [10–12]. The mammalian enzymes consist of multiple N-terminal dsRNA-binding domains (dsRBDs) followed by the C-terminal deaminase domain. Their dsRBDs bind to manifold dsRNA structures yielding editing at tens of thousands up to millions of sites in the human transcriptome [13–16]. The main challenge of all RNA editing approaches is to obtain high efficiency (ideally in any sequence context) while keeping editing restricted to the targeted site [17••,18•,19••]. This is particularly true when a known hyperactive mutant [20••], called E/Q, is applied to improve efficiency and codon scope. Here, we focus on three strategies that apply fusion proteins comprising a human ADAR deaminase domain combined with a targeting domain that enables addressing of the fusion with a gRNA (Figure 1). The gRNA determines on-target specificity in a simple and programmable way, by putting the targeted adenosine into an RNA duplex, preferably into mismatch with cytidine [21].

SNAP–ADARs

SNAP–ADARs were pioneered in our lab [22••]. It was the first strategy published that applies an engineered fusion for site-directed RNA editing. The ribonucleoprotein assembly strategy is uncommon and requires explanation (Figure 1). The SNAP-tag is a self-labeling protein (~200 aa) evolved from the human O^6-alkylguanine-

Figure 1



RNA-guided deaminases engineered for site-directed A-to-I RNA editing. During engineering, the deaminase domain of a human ADAR (ADAR-D, shown in blue) was fused to a protein domain (shown in purple) that enables the targeting of the fusion with a guide RNA (gRNA, shown in black). The gRNA directs the engineered fusions to the target RNA (shown in gray) to form an RNA duplex, typically containing an A:C mismatch at the target site, to induce site-specific deamination. The assembly of editase and gRNA is mediated either by covalent bond formation (SNAP–ADAR/BG-gRNA) or by non-covalent interactions (λN-ADAR/boxB-gRNA, dCas13b-ADAR/DR-gRNA). The relative sizes of the respective fusion proteins and their gRNAs are roughly represented in the figure.

DNA alkyltransferase [23•,24]. It allows to covalently bind to a tailored gRNA in a defined 1:1 stoichiometry, given the gRNA carries a chemical tag, O^6-benzylguanine (BG). The resulting gRNA-deaminase conjugates are quickly formed *in vitro* and *in vivo* [22••,25••,26••]. Due to the need for the BG-moiety, the gRNA component is not genetically encodable. This represents the main disadvantage of the system. In situations where the installment of a permanent correction is required, for example, restoration of an inherited loss-of-function mutation, this might be a serious drawback. However, the short gRNAs (ca. 20 nt) are very readily transfected into a broad range of cells, similar to siRNAs [27] and antagomirs [28]. The quick introduction of transient changes at many sites into the transcriptome might be a strength of the approach. Tailored chemical modification of the gRNA (*2′-O*-methylation and phosphorothioate) allows to further optimize their properties in terms of potency, specificity, efficiency, stability, and probably also immunogenicity [29,30].

The strategy is currently the best characterized. SNAP–ADARs are particularly effective under genomic integration (single copy) of the editase and achieve very high editing yields with broad substrate scope on endogenous targets. Specifically, preferred codons in the ORFs of endogenous GAPDH, GUSB, KRAS and STAT1 transcripts were edited efficiently (50–90% yield) with short gRNAs (*22* nt) [19••]. To assess the full codon scope, all 16 codons (5′-NAN) have been tested in an endogenous transcript, 11/16 codons could be edited in yields >50%. These contain several highly attractive substitutions, including Thr-to-Ala, Lys-to-Arg, Tyr-to-Cys, and Ser-to-Gly. Notable is also the speed and duration of the effect. The maximum editing was achieved 3 h after transfection of the gRNA and lasted over several days.

The dense chemical modification and the shortness of the gRNA enable to suppress off-target editing inside the gRNA/mRNA duplex, even if highly A-rich codons like 5′-AAA or 5′-CAA are addressed [19••]. Wildtpye SNAP–ADARs show very little global off-target editing. However, the hyperactive SNAP–ADAR E/Q mutants, required to achieve a broad substrate scope, shows a moderate number of off-target edits. It needs to be determined how problematic this is. Further lowering of the fusion expression may further reduce off-target editing [19••].

Finally, SNAP–ADARs have been applied *in vivo* in *Platynereis dumerilii* [26••]. The strategy also allows to include photocontrol of the gRNA/deaminase assembly based on the photocaging of the BG moiety. This has been demonstrated in cell culture and *in vivo* [26••]. We have shown that RNA editing can be used to add whole protein stretches to the N-termini and C-termini by the editing-induced inclusion of alternative start and stop codons in the 5′-UTRs and 3′-UTRs, respectively [31•].

λN-ADARs

The λN-ADAR strategy applies the classical (however trans-acting) tethering approach for the (non-covalent) assembly of the gRNA-deaminase complex (Figure 1). It makes use of the well-known interaction between the boxB RNA hairpin and the *22* aa-long λN peptide [32]. The group of Joshua Rosenthal pioneered this approach and demonstrated the restoration of CFTR function by targeting the λN-ADAR2 protein to a premature stop codon in a co-injected CFTR transcript in Xenopus oocytes [33••]. The editing efficiency in cell culture has been remarkably enhanced by applying the hyperactive ADAR2 E/Q mutant and by fusing it to up to four λN peptides (4λN-ADAR2 E/Q). Together with a gRNA containing two boxB hairpins (∼85 nt), and expressed from a U6-promotor, editing levels up to 80% were achieved for preferred codons [34•]. A full characterization of the codon scope of this system is not available, however, a similar scope as the SNAP–ADAR system is