Emerging applications of catalytically inactive CRISPR-Cas13 system in mRNA engineering

Yan-Hua Chen1, Qian-Qian Zhou1, Fu-Wen Yuan1*

1Academy of Integrative Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China.

Abstract
Posttranscriptional regulations of different types of RNA, including rRNA, tRNA, mRNA and ncRNA are widely involved in normal physiology and diseases. mRNA, as the intermediary product between gene and protein, whose posttranscriptional regulations such as alternative splicing, alternative polyadenylation and modifications impact its coded protein expression and functions. However, the functional significance and therapeutic potential of RNA posttranscriptional regulations are not well studied due to the lack of suitable RNA engineering platforms. The discovery of a novel CRISPR-Cas system termed CRISPR-Cas13 in 2015 that specifically targets RNA templates brought a new role to CRISPR to target and edit RNA with high specificity, which opened a new era of RNA manipulations to some degree. This review will summarize the emerging applications of the catalytically inactive CRISPR-Cas13 system (CRISPR-dCas13) in mRNA engineering and highlight the prospection of the CRISPR-dCas13 system for other RNA modification regulations and its therapeutic potential.

Keywords: CRISPR-dCas13; RNA posttranscriptional regulations; alternative splicing; alternative polyadenylation; m6A modification; base editing
Background

CRISPR-Cas13, including CRISPR-Cas13a, CRISPR-Cas13b, CRISPR-Cas13c, CRISPR-Cas13d, CRISPR-Cas13x and CRISPR-Cas13y, belongs to the type VI CRISPR-Cas system [1–8]. Different from the previous prevalent CRISPR-Cas9, which targets and cleavages the double-strand DNA, type VI (CRISPR-Cas13) systems provide potent RNA recognizing and targeting capability and do not introduce unwanted and irreversable DNA changes as well [2, 9, 10]. Like CRISPR-Cas9 systems, CRISPR-Cas13 systems use a single signature Cas protein (Cas13), which is also a “Bilobed” effector that consists of one crRNA “Recognition” lobe that helps in binding and recognizing crRNA, and one “Nuclease” lobe consists of two higher eukaryotic and prokaryotic nucleotide-binding domains that responsible for deoxirribonuclease cleavage [1, 11]. In addition to the effector protein, crRNA is also required for the recognition and cleavage of target single-strand RNA, which is a 64–66nt RNA sequence constituted with a 24–30nt spacer region and a direct repeat sequence [1, 11]. Due to the high specificity and RNA targeting efficiency, catalytically active CRISPR-Cas13 systems showed great promise for the treatment of diseases, such as cancer and neurological disease by targeting disease-relevant genes in vivo and in vitro [2, 9, 12, 13]. Moreover, CRISPR-Cas13 systems are able to adapt to RNA sensors using its collateral cleavage activity that cleaves the target nucleotide sequence followed by the cleavage of neighboring non-target sequences, which was employed as a “switch” to turn on various types of reporter molecules, such benefit has been showcased in the development of CRISPR-Cas13-based detection platforms, such as Specific High-Sensitivity Enzymatic Reporter un-LOCKing and CRISPR/CAS-based Colorimetric nucleic Acid DETection, which can detect RNA (especially SARs-CoV-2) specifically and sensitively [14–23]. In addition to the RNA cleavage activity, accumulating studies demonstrated the potential of the engineered catalytically dead CRISPR-Cas13 (CRISPR-dCas13) systems in mRNA modulation, such as mRNA alternative splicing (AS), alternative polyadenylation (APA), RNA modifications and in vivo RNA localization tracing.

CRISPR-dCas13 for AS/APA manipulation

CRISPR-dCas13 for AS engineering

AS, which allows the expression of different mRNA isoforms from a single gene, is one of the fundamental processes in pre-mRNA maturation and occurs in over 90% of human genes, resulting in gene expression and proteome diversity [24, 25]. Dysregulation of AS is frequently observed and profoundly disrupts normal cellular processes in multiple diseases, such as cancers, aging and diabetes [36–32]. Konermann et al. showed that CRISPR-dCas13d or engineered fusions to the Gly-rich C-terminal domain of hmRNPa1, one of the most abundant hnRNP family, targeting the splice elements could successfully perturb the exon exclusion or inclusion in the reporter system and endogenous genes, which was proved to be a useful tool for functional studies of AS events in later studies [2, 33]. Importantly, delivery of AAV1 encapsulated dCas13x and gRNA targeting microtubule associated protein tau exon 10 could induce exon exclusion to alleviate dysregulated 4R/3R tau ratios in patient-derived human-induced pluripotent stem cells, which was previously reported to induce the progression of neurodegeneration [34]. More recently, Du et al. developed a CRISPR artificial splicing factors (CASFx) by fusing RNA-target dCas13 with splicing regulatory domains such as N-terminal domains of splicing factor RBFOX1, which can induce simultaneous exon inclusion and skipping at distinct targets by differential positioning of CASFx (Figure 1A) [35]. It also successfully activated the endogenous SMM2-E7 inclusion in GM03813 fibroblast cells derived from a type ll SMA patient. Collectively, CRISPR-dCas13-based AS manipulation tools enabled the AS regulation in endogenous AS events and showed great potential to address disease-related AS events in patients [36].

Figure 1 dCas13-based platforms for AS/APA manipulations. (A) Pre-RNAs harbor alternative splicing site (ss) may undergo alternative splicing and generate different transcripts in diseases. dCas13 or CASFx targeting the desired ss can block the access of AS factors and perturb the splicing process. (B) In some conditions, such as cancers, genes with multiple 3'UTR PAS sites prefer to use the proximal PAS, thus generating transcripts with shorter 3'UTR. dCas13 targeting the pPAS represses pPAS usage and generates longer 3'UTR isoforms. AS, alternative splicing; APA, alternative polyadenylation; CASFx, CRISPR artificial splicing factors; PAS, polyadenylation site; pPAS, proximal polyadenylation site; dPAS, distal polyadenylation site; CDS, coding sequence.
CRISPR-dCas13 for APA regulation

Different from AS, APA does not always change the protein-coding. Four types of APA have been reported, among which, tandem 3’UTR APA accounts for over 50% of all APA events as we reviewed before, which affects the abundant cis-elements that can be targeted by miRNA or RNA binding and resulting in changes of mRNA stability, localization, translation and protein localization [37]. Besides, the most straightforward result of different APA types occurs within the protein-coding region alters the amino acid sequence and generates a different protein isoform [38, 39]. However, as the sequence of shortened isoforms generated by the usage of proximal polyadenylation site (PAS) is always covered by lengthened isoforms, previously tools could not directly target and manipulate different APA isoforms specifically. To character the APA manipulation potential of CRISPR-Cas13 systems, Tian et al. screened a panel of dCas13 proteins, including LbadCas13a, PgudCas13b, RandCas13b, EdsCas13d, AdmdCas13d and RfxdCas13d with an APA reporter, which revealed that CRISPR-dPgpuCas13b system has the most significant APA manipulation efficiency, which can also engineer the endogenous PAS usage and is an efficient APA perturbation tool for investigating the disease-relevant APA events (Figure 1B) [40]. It’s worthy to note that a CRISPR-dCas9-based APA manipulation platform termed CRISPRPas was also reported by another group, which can enhance the proximal PAS usage by using a dCas9 and coupling its target site with PAS [41, 42]. These tools epigenetically regulate the PAS usage without changing DNA sequence compared with the conventional Cas9-mediated gene editing of PAS [43, 44].

CRISPR-dCas13 for RNA modification

Internal mRNA modifications are frequently observed and play diverse biological processes. They are crucial for normal physiology and pathophysiological states, among which, N6-methyladenosine (m6A) and Ψ are most prevalent with approximately 0.5% of the respective bases carrying modifications [45, 46]. These modifications influence the outcome of transcripts by regulating mRNA AS, translocation, translation and stability [47–50]. m6A is currently the best-characterized and most abundant mRNA epitranscriptomic mark so far since it was first discovered in 1974 as the major form of internal methylation within eukaryotic mRNA, which can change the mRNA translation efficiency and mRNA stability [51–53]. Dysregulation of m6A is reported in different diseases, including cancers, osteoarthritis, cardiovascular diseases and musculoskeletal disorders [54–57]. Despite a growing appreciation of the importance of m6A in biology and disease, current understanding of its specific functions continues to lag far behind DNA and protein modifications because of the lack of methods to manipulate the m6A specifically.

The mammalian mRNA mA is written by writers (e.g., METTL3 and METTL14), recognized by readers (e.g., YTHDF1 and YTHDF2) and erased by erasers (e.g., FTO and ALKBH5) [53]. Previous studies of mA’s biological processes mainly by perturbing the expression of mA regulators, which, however, induced thousands of transcripts’ methylation state changes. By fusing the METTL3 zinc finger RNA-binding motifs (M3) and METTL3-interacting domain of METTL14 (M14) to dPsgCas13b, Wilson et al. developed an mA installation tool that is able to enhance the endogenous mA modification on the desired mRNA site with high specificity and efficiency [58]. Meanwhile, another group generated an mA demethylation platform termed dM6ACRISPR by linking a dPsgCas13b to mA demethylase AlkB homolog 5, which incurs efficient demethylation of targeted transcripts with limited off-target effects [59]. Moreover, after fusing the erase the Fat mass and obesity-associated protein and writer M3M14 Msase to dCas13b, respectively, Zhao et al. successfully manipulate the endogenous RNA mA erase and installation. With heterodimerization of CBP and a truncated version of light-sensitive protein CB11 and CR2PHR, the photolyase homology region of CR2Y, they further engineered a photoactevatable RNA mA editing system, which offers an appealing avenue to control RNA manipulation remotely [60]. These studies together provide programmable and in vivo engineering tools to study specific mRNA methylation and demethylation and their biological functions, which also offered proof of concept of CRISPR-dCas13 systems for other types of RNA modification manipulation, such as Ψ and mA.

CRISPR-dCas13 mediated RNA base editing

There are over 3,200 human pathogenic single nucleotide polymorphisms have been identified in the ClinVar database, over 60% of which are A>T to C>G and C>G to T>A mutations [61]. Gene editing approaches that address these pathogenic variants in human cells have the potential to advance our understanding of the related genetic disease, which could also enable new therapeutics [62, 63]. Previous base editors usually indirectly modify RNA transcripts by editing the DNA nucleotide, which may induce irreversible off-target edits [62, 63]. By linking the adenosine deaminase enzymes that mediate endogenous editing of transcripts via hydrolytic deamination of adenosine to inosine (a nucleobase that is functionally equivalent to guanosine in translation and splicing) to dCas13a, Cox et al. developed the first CRISPR-Cas13-based RNA base editing platform termed RNA editing for programmable A to I replacement (REPAIR), that can convert adenosine to inosine in mammalian cells that maintains its high efficiency on target editing for endogenous RNA single base manipulation [4, 64, 65]. Liu et al. further engineered this A to I replacement system by embedding ADAR2DD into dCasRxt’s flexible loop instead of being linked at its terminal. Such a new editor, dubbed REPAIRx, is precise yet highly efficient, outperforming various previous versions on both mRNA and nuclear RNA targets, which markedly expands the RNA editing toolkit and illustrates a novel strategy for base editor optimization [66]. In addition to the CRISPR-dCas13-based A to I RNA editing approach, the same group also reported a programmable C to U RNA editing approach by fusing dCas13 with a cytidine deaminase referred to as RNA editing for specific C to U exchange [67]. This platform doubles the number of mutations targetable by RNA editing. It enables modulation of phosphosigalning-relevant residues, which also enables multiplexed C-to-U and A-to-I editing using the tailored guide RNAs [67].

Perspectives

As an emerging CRISPR-Gas system, the high specificity and RNA targeting efficiency enable CRISPR-Cas13 systems to become promising toolkits for RNA engineering. CRISPR-dCas13, which lost the nuclease activity was recently proved to be an effective RNA manipulation platform in APA/AS perturbation, mA modification and base editing. While most of the current CRISPR-dCas13-based RNA manipulation tools, such as CASFx for APA regulation, REPAIR and RNA editing for specific C to U exchange for base editing and the CRISPR-dCas13-based mA modification tools are either not specific or not effective enough in some degree [33, 35, 58, 66]. Further studies are needed to overcome these concerns and to explore the potential of CRISPR-dCas13-based therapeutics. What’s more, the development of novel CRISPR-dCas13-based technologies for other types of RNA engineering is also worthy of further exploration, such as the RNA localization tracing in vivo, regulation of RNA Ψ and mA modifications and RNA short sequence deletion or insertion [10, 68, 69, 70].

References


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