RNA-Targeting CRISPR–Cas Systems

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Many CRISPR–Cas systems have been used as a backbone for the development of potent research tools, with Cas9 being the most widespread. While most of the utilized systems are DNA-targeting, recently more and more attention is being gained by those that target RNA. Their ability to specifically recognize a given RNA sequence in an easily programmable way makes them ideal candidates for developing new research tools.

CRSIPR/Cas Cas9 Cas13 RNA Cmr Csm

1. Introduction

"Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)–CRISPR-associated (Cas) (CRISPR–Cas) systems originate from Prokaryotes, where they serve primarily as a defensive mechanism against mobile genetic elements like phages and plasmids [1]. These systems consist of two components: genomic locus called CRISPR and Cas proteins. CRISPR array contains a series of short sequences of foreign origin called spacers that enable recognition of specific mobile genetic elements (MGEs) that were previously encountered flanked by a leader sequence [2][3][4][5]. The second component- Cas proteins are coded by cas genes, usually located in the proximity of a CRISPR array. Cas proteins play an effector role in CRISPR–Cas systems.

The mechanism of CRISPR–Cas systems consists of three phases: adaptation, maturation, and interference. During the adaptation phase, new spacers are incorporated into the CRISPR array into its leader end [6]. During the maturation phase the CRISPR array is transcribed. The resulting transcript called pre-CRISPR RNA (pre-crRNA) is further processed into a set of CRISPR RNA (crRNA) molecules, each containing a single spacer flanked by fragments of a repeat sequence [7][8][9][10][11][12]. Subsequently, crRNAs are incorporated into ribonucleoprotein (RNP) complexes together with Cas proteins. RNP complexes scan nucleic acids searching for a sequence complementary to that encoded by crRNA [13]. Recognition of such a sequence triggers the interference phase that leads to degradation of a recognized nucleic acid [14][15][16].

Discovery of the CRISPR–Cas systems has revolutionized modern molecular biology. Due to their unique mechanism, they possess several features highly desirable for potential tools in this field. First of all, they are highly specific in terms of recognized sequence. Furthermore, this specificity can be easily altered by modifying the sequence coding the crRNA. The modular structure of the CRISPR locus makes these modifications even easier. The range of applications for CRISPR–Cas systems can be further expanded by modifying the Cas proteins

themselves [17]. Most of the CRISPR–Cas systems described to date are those targeting DNA sequences. In recent years several new types of CRISPR–Cas systems targeting RNA were discovered, paving the way for the development of new tools for research and biotechnology." [18]

2. Overview of RNA-Targeting CRISPR–Cas Systems

2.1. Type III (Cmr/Csm) Systems

The effector complexes of type III systems consist of multiple subunits and thus they belong to the class 1 CRISPR-Cas systems. Maturation of crRNAs in majority of type III systems includes two stages: first pre-crRNA is cleaved by Cas6, then resulting crRNAs are further trimmed in a mechanism that has not been fully described yet. A unique feature of type III CRISPR-Cas systems is that they use three nuclease activities (Fig. 1). The first one is a sequence specific RNA cleavage performed by the Cas7, which is a integral part of the RNP complex [19][20] (Fig. 1a). The second nuclease activity of this type is Cas10-dependent non-specific ssDNA cleavage [21]. This activity requires transcription to occur. Once the RNA polymerase opens the DNA double helix it exposes the antisense strand. Digestion of the antisense strand is triggered by the complementarity between crRNA and the targeted RNA [22]. The third nuclease activity of type III CRISPR/Cas systems is a non-specific RNA degradation. This activity also depends on Cas10, but its mechanism is different. In this case so called palm domain of Cas10 is involved in conversion of ATP into cyclic oligoadenylates [23][24]. Conversion is non constitutive and it is triggered by binding of the RNP complex with targeted RNA with simultaneous non-complementarity between crRNA handle and targeted RNA. These cyclic oligoadenylates allosterically activate the Csm6/Csx1 (depending on a given system subtype) proteins, which are responsible for RNA cleavage, yet they are not incorporated into RNP complexes themselves.

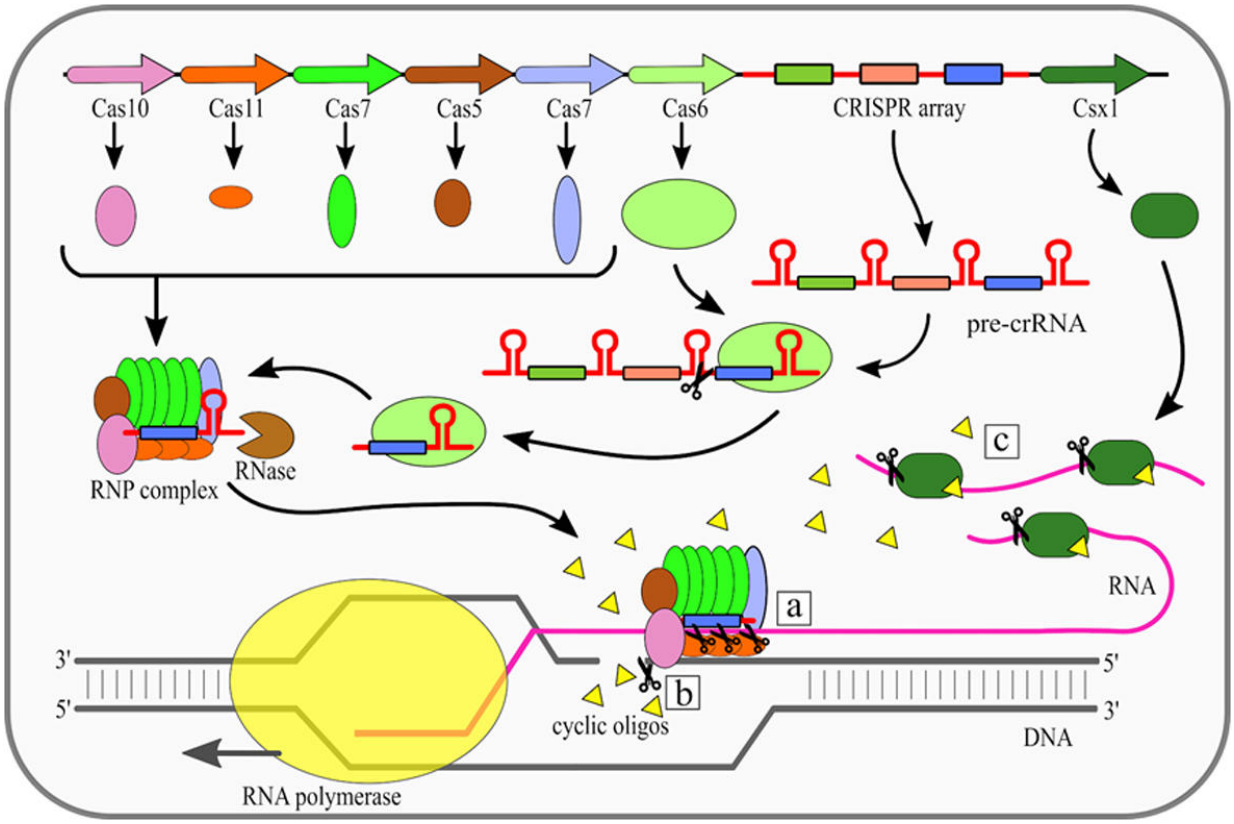

Figure 1. Mechanism of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)–CRISPR-associated (Cas) type III (Csm/Cmr) system. Cas6 endoribonuclease cleaves pre-CRISPR RNA (pre-crRNA) within the repeat region. Subsequently, the ribonucleoprotein (RNP) complex is assembled, while the 3' end of crRNA is trimmed by an unknown nuclease. There are three nuclease activities of the RNP complex: (a) specific RNA cleavage, (b) non-specific ssDNA cleavage, (c) non-specific RNA degradation. [18]

2.2. Type VI (Cas13) Systems

The type VI CRISPR-Cas systems present a relatively simple structure, as they require only one Cas13 protein and crRNA molecule (Fig. 2). Cas13 performs the primary processing of pre-crRNA itself [25]. Secondary crRNA processing is performed presumably by other host nucleases. What is interesting, it was shown that the type VI nucleolytic activy can be maintained even when non processed crRNA molecules are used [26]. The intereference mechanism of this type includes conformational change of Cas13 protein, which is induced by compelementarity between crRNA and targeted ssRNA. Upon that change, two HEPN domains are moved closed together to form a single catalytic site. As this site is exposed outside of the Cas13 it cleaves not only targeted ssRNA, but also any other ssRNA in the vicinity. This phenomenon is known as a collateral damage of Cas13 system [17].

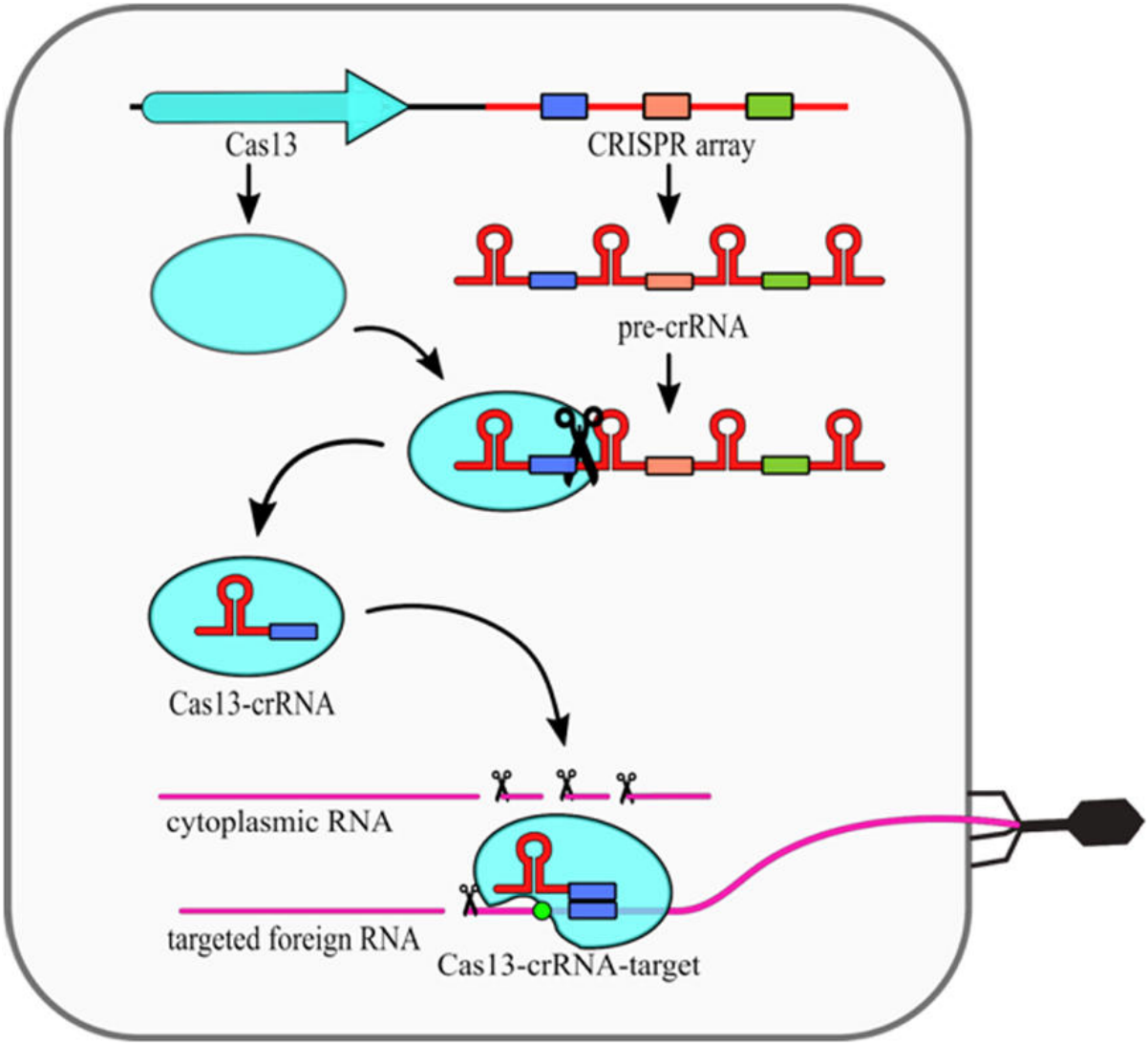

Figure 2. Mechanism of the CRISPR–Cas13 (type VI) system. The CRISPR array is transcribed into a long pre-crRNA transcript, which is subsequently processed into mature crRNAs by Cas13 protein. The crRNA-Cas13 complex scans the ssRNA searching for protospacer. Complementarity between crRNA and the protospacer sequence together with the presence of Protospacer Flanking Sequence (PFS) (green circle) induces conformational changes of Cas13, which results in higher eukaryotes and prokaryotes nucleotide binding (HEPN) domains activation and their displacement to the protein surface. This results in nonspecific RNA cleavage. [18]

2.3. Type II (Cas9) Systems

In addition to CRISPR array and Cas proteins type II CRISPR/Cas systems encode additional trans activating RNA (tracrRNA), which mediates interaction between crRNAs and Cas9. The primary processing of type II pre-crRNAs is performed by RNase III [9]. Fragment of tracrRNA binds to a repeat sequence creating a duplex. This dulex is then cleaved by RNase III [8]. Subsequently, this intermediate crRNA is further trimmed by an unknown nuclease. The maturated crRNA:tracrRNA duplex is incorporated into Cas9. Similar to type III systems, Cas9 was shown to possess several nucleolytic activities (Figure. 3). The most common nuclease activity among type II systems is specific dsDNA cleavage (Figure. 3a). For some type II CRISPR/Cas systems additional nucleolytic directed

against RNA have been described. In *Francisella novicida* type II encodes additional small RNA named scaRNA, which is supposed to replace crRNA in the RNP complex. This enable it to target RNA instead of DNA [27]. Recent studies have shown that some of the type II CRISPR-Cas systems present natural nucleolytic activity against ssRNA targets. To date, this particular ssRNA targeting activity has been described for *Staphylococcus aureus* [28], *Campylobacter jejuni* [29], and *Neisseria meningitides* [30]. However, still very little is known about mechanistic details of this activity.

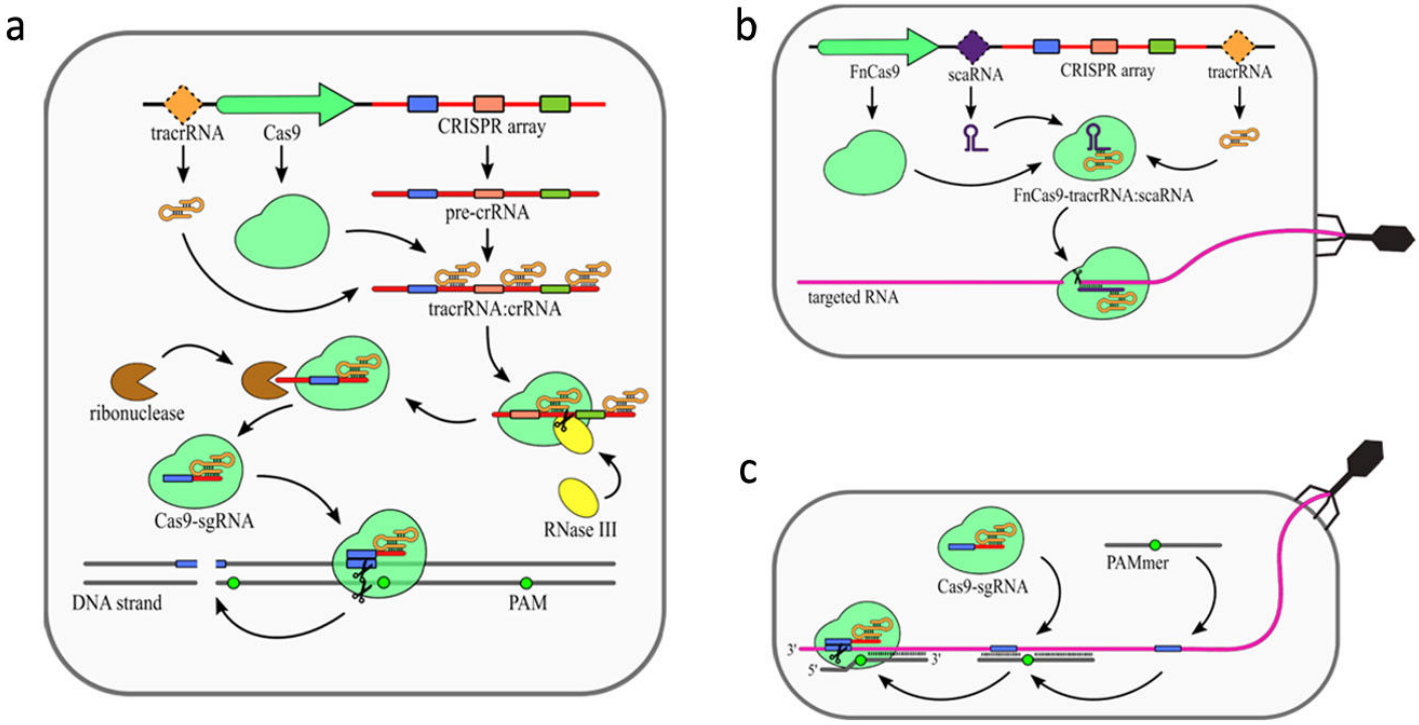

Figure 3. Mechanism of the CRISPR–Cas9 (type II) system. (a) DNA targeting CRISPR array transcription generates pre-crRNA. Maturation of the crRNAs is dependent on trans activating RNA (tracrRNA), which is partially complementary to the repeat sequences in the pre-crRNA resulting in tracrRNA/crRNA duplex formation. Those duplexes are bound and stabilized by Cas9 protein. Host RNase III is then recruited to cleave pre-crRNA into units containing single spacer sequences. Further trimming of the crRNAs is performed by unknown ribonuclease. The complex of Cas9 and single guide RNA (sgRNA: tracrRNA–crRNA) scans DNA until it finds a Protospacer-Associated Motif (PAM) sequence. The DNA strand is then unwound, allowing sgRNA for complementarity verification. Positive recognition results in cleavage of both DNA strands. (b) scaRNA-dependent RNA targeting was observed for Cas9 from *Francisella novicida* (FnCas9). Small CRISPR/Cas-associated RNA (scaRNA) hybridizes with tracrRNA to form heteroduplex that binds Cas9 protein. FnCas9–tracrRNA/scaRNA complex targets RNA partially complementary to scaRNA sequence. The precise mechanism of FnCas9 remains unclear. (c) PAM-presenting oligonucleotide (PAMer)-dependent RNA targeting Functional Cas9–sgRNA complex is able to target RNA in the presence of PAMmers–short DNA oligonucleotides containing PAM. When the PAMmer is bound to target RNA, the Cas9–sgRNA complex is able to recognize and cleave the RNA as long as complementarity between sgRNA and targeted RNA is maintained. [18]

4. Applications Based on RNA-Targeting CRISPR–Cas Systems

Table 1. Applications based on RNA-targeting CRISPR-Cas systems. [18] modified.

Application	CRISPR-Cas type (technology name)	Reference
RNA knockdown	Cas9	[28]
	Cas13a/b/c	[31][32][33][25][34]
	Csm/Cmr	[35][36]
	Cas13d (CasRx)	[37]
RNA imagingand tracking	dCas9	[38]
	dCas13a	[33]
RNA editing	Cas13b (REPAIR)	[39]
nucleic acid detection	Cas13a (SHERLOCKv1)	[40]
	Cas13 + Csm6 (SHERLOCKv2)	[41]
splicing alteration	dCas13d (dCasRx)	[35]
resistance againstRNA viruses	FnCas9	[42][43]
	Cas13a	[19]

induction of apoptosis	Cas13a	[17]
regulation of gene expression	Cas13b	[19]
specific RNA isolation	dCas9	[44]
elimination of repetitive sequences	Cas9	[20]

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