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Modified Cas (CRISPR-associated proteins) for Genome Editing and Beyond

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Abstract

As one of the most popular tools for genome editing, CRISPR/Cas9 system has been widely used in gene targeting, transcriptional regulation, epigenetic modification, even in gene therapy and drug delivery. Although CRISPR/Cas9 system provides a simple, specific and high efficient platform, it still has some limitations. Recently, several modified versions of CRISPR/Cas systems (Cpf1, C2c2, dCas9 and dC2c2, et al.) have been remarkably developed for more powerful and customizable genome editing and function studies. In this review, we summarized the key findings and progress on Cas9 and modified Cas proteins, which would help to better understand CRISPR/Cas systems and advanced future studies on their applications.

Keywords: CRISPR/Cas system; Cas9; Modified Cas; Cpf1; C2c2; dCas9; dC2c2; Genome editing; Transcriptional regulation; Epigenetic modification

Introduction

Recent years have seen tremendous progress in genome editing tools, which have been widely applied in facilitating the functional investigation of specific genes or mutations, transcriptional regulation, epigenetic modification, even gene therapy and drug delivery [1-4]. Comparing with ZFNs (Zinc finger nucleases) and TALENs (Transcription activator-like effector nucleases) [5], CRISPR/Cas (Clustered regularly interspaced short palindromic repeats/CRISPR associated) systems have become one of the most popular tools due to their simplicity, high efficiency and versatility (Table 1).

CRISPR/Cas systems were found in adaptive immune systems from 50% of bacteria and 90% of archaea [6]. A typical CRISPR/Cas9 system contains two components Cas9 protein and SgRNA (single guide RNA) (Table 2). SgRNA is consisted of CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA) [7]. gRNA directs Cas9 protein to target DNA through recognizing PAM (Protospacer Adjacent Motif) sequence. In CRISPR/Cas9 system, Cas9 targets and cleaves DNA at the site PAM sequence is 5'-NG/AG [8]. During the last four years, development on modified CRISPR/Cas systems has remarkably broadened the applications in scientific and clinical fields [2-4]. In this review, we summarized the progress on modified Cas proteins as well as their applications (Table 1).

Cpf1 belongs to the type V CRISPR/Cas systems. Cpf1 has the similar endonuclease function as cas9 protein [9] (Table 2). Comparing with typical CRISPR/cas9 system, CRISPR/Cfp1 system has three distinct characteristics. Firstly, to target DNA, only one crRNA is required in CRISPR/Cfp1 system while both crRNA and tracrRNA are required in CRISPR/Cas9 system [10,11]. Thus, the design of SgRNA is much easier in CRSIPR/Cpf1 system. Secondly, CRISPR/Cpf1 system is a short T-rich PAM rather than the G-rich PAM in typical CRISPR/Cas9 system. Therefore, the genome regions for Cpf1 protein targeting and DNA cleaving are not limited in G-rich regions, which offer more genome editing-sites options. Thirdly, Cpf1-crRNA complex cleaves target DNA and creates a staggered DNA double-stranded break [9]. Thus, Cpf1 generates a sticky ends of DNA cleavage break rather than the blunt ends by Cas9 protein [7,12,13], which is appropriate for gene insertion by non-homologous end joining (NHEJ) in the mammalian genome [14]. Recent crystal structure demonstrated the striking similarity and significant differences between Cpf1 and Cas9 [15].

C2c2 is one effector protein of class 2 type VI systems (Table 2). Although C2c2 lacks known homology domain of DNA nuclease, it functions as an RNase due to containing two HEPN (Higher Eukaryotes and Prokaryotes Nucleotide-binding) domains [16,17]. Thus, different from other RuvC domain-containing Cas proteins, CRISPR/C2c2 system can effectively cleave RNA. Only one crRNA is required for RNA cleavage in CRISPR/C2c2 system. Then, C2c2 cleaves ssRNA targets which contain complementary protospacers. CRISPR/C2c2 system prevents sequence-specific mRNA in a RNA-guiding manner [17]. Up to date, CRISPR/C2c2 system has become one of the most efficient tools for RNA editing.

Better understanding of mechanisms of Cas proteins helps to explore their potential applications. Two modified Cas, dCas9 (deficient Cas9) and dC2c2 (deficient C2c2), were successfully used in transcriptional regulation and epigenetic modification (Table 1) [18]. dCas9, a catalytically inactive Cas9, losses its endonuclease activity due to carrying a H84A mutation and a D10A mutation [19]. However, it still remains its DNA binding ability in a sequence-specific manner. Also, dCas9-fusion protein can be activator, repressor or epigenetic modular via fused to other effector domains. For example, dCas9-gRNA complex could decrease gene expression through disturbing the process of transcription elongation, RNA polymerase or transcription factor binding [20]. dcas9-KRAB (Kruppel-associated box) can enhance the ability of transcriptional inhibition. dCas9-VP16 or dCas9-VP64 complex can promote gene expression through fused with activation domains VP16/VP64 [21-24]. Moreover, dCas9 has been developed to be a power tool for epigenome editing. p300 core, which is the core domain of HAT (Histone Acetyltransferase), is fused to dCas9 to catalyze the addition of a H3K27 acetylation mark. Then dCas9-p300 fusion protein can activate gene expression through targeting promoter, proximal enhancers and distal enhancers [25]. dC2c2, a catalytically inactive C2c2, losses its endoribonuclease activity due to a mutation

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Name	Type	Nuclease/Endoribonuclease Activity	Complexity	Function	References
Cas9	Type II	Yes	Middle	SgRNA-guided targeting DNA	[7]
Cpf1	Type V	Yes	Simple	CrRNA-guided targeting DNA	[9]
C2c2	Type VI	Yes	Simple	Targeting RNA	[17]
dCas9	Type II	No	Middle	Transcriptional regulation, tracking DNA or RNA	[20]
dC2c2	Type VI	No	Middle	Transcriptional regulation, tracking RNA	[17]

Table 1: Cas9 and modified Cas proteins.

System	Brief Introduction
CRISPR/Cas9	CRISPR/Cas9 system is class 2 type II system from *Streptococcus pyogenes*. It contains two components Cas9 protein and SgRNA. gRNA directs Cas9 protein to target DNA through recognizing PAM sequence.
CRISPR/Cpf1	CRISPR/Cpf1 system is class 2 type V system from Acidaminococcus and Lachnospiraceae. It consists of Cpf1 protein and crRNA. crRNA directs Cpf1 protein to target DNA through recognizing PAM sequence.
CRISPR/C2c2	CRISPR/C2c2 is class 2 type VI system from the bacterium *Leptotrichia shahii*. It consists of C2c2 protein and crRNA. crRNA directs C2c2 protein to target RNA.

Table 2: Introduction of three CRISPR Cas systems.

in its HEPN domain. dC2c2 can be used to target sequence-specific mRNAs to regulate their functions. In addition, dC2c2 can also be fused with fluorescent protein to track specific RNAs localization [17].

Conclusion

CRISPR/Cas systems have been widely used in genome editing, transcriptional and epigenetic regulation [1,26,27]. Rapid development in CRISPR/Cas techniques has brought new revolution in genome editing and beyond. Just as Heidi Ledford said, "CRISPR: Gene editing is just beginning" [26]. Progress on Cas9 and modified Cas proteins will greatly extend their applications.

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References

1. Vora S, Tuttle M, Cheng J, Church G (2016) Next stop for the CRISPR revolution: RNA-guided epigenetic regulators. FEBS J.
2. LaFountaine JS, Fathe K, Smyth HD (2015) Delivery and therapeutic applications of gene editing technologies ZFNs, TALENs, and CRISPR/Cas9. Int J Pharm 494: 180-194.
3. Zhang Y, Yin C, Zhang T, Li F, Yang W, et al. (2015) CRISPR/gRNA-directed synergistic activation mediator (SAM) induces specific, persistent and robust reactivation of the HIV-1 latent reservoirs. Sci Rep 5: 16277.
4. Limsirichai P, Gaj T, Schaffer DV (2016) CRISPR-mediated Activation of Latent HIV-1 Expression. Mol Ther 24: 499-507.
5. Wood AJ, Lo TW, Zeitler B, Pickle CS, Ralston EJ, et al. (2011) Targeted genome editing across species using ZFNs and TALENs. Science 333: 307.
6. Makarova KS, Wolf YI, Alkhnbashi OS, Costa F, Shah SA, et al. (2015) An updated evolutionary classification of CRISPR-Cas systems. Nat Rev Microbiol 13: 722-736.
7. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, et al. (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337: 816-821.
8. Heler R, Samai P, Modell JW, Weiner C, Goldberg GW, et al. (2015) Cas9 specifies functional viral targets during CRISPR-Cas adaptation. Nature 519: 199-202.
9. Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KM, et al. (2015) Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell 163: 759-771.
10. Chylinski K, Le Rhun A, Charpentier E (2013) The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems. RNA Biol 10: 726-737.
11. Deltcheva E, Chylinski K, Sharma CM, Gonzales K, Chao Y, et al. (2011) CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. Nature 471: 602-607.
12. Garneau JE, Dupuis ME, Villion M, Romero DA, Barrangou R, et al. (2010) The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature 468: 67-71.
13. Gasiunas G, Barrangou R, Horvath P, Siksnys V (2012) Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl Acad Sci USA 109: E2579-2586.
14. Maresca M, Lin VG, Guo N, Yang Y (2013) Obligate ligation-gated recombination (ObLiGaRe): custom-designed nuclease-mediated targeted integration through nonhomologous end joining. Genome Res 23: 539-546.
15. Yamano T, Nishimasu H, Zetsche B, Hiran H, Slaymaker HI, et al. (2016) Crystal Structure of Cpf1 in Complex with Guide RNA and Target DNA. Cell 165: 949-962.
16. Shmakov S, Abudayyeh OO, Makarova KS, Wolf YI, Gootenberg JS, et al. (2015) Discovery and Functional Characterization of Diverse Class 2 CRISPR-Cas Systems. Mol Cell 60: 385-397.
17. Abudayyeh OO, Gootenberg JS, Konermann S, Joung J, Slaymaker IM, et al. (2016) C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science 353: aaf5573.
18. Kiani S, Chavez A, Tuttle M, Hall, Chari R, et al. (2015) Cas9 gRNA engineering for genome editing, activation and repression. Nat Methods 12: 1051-1054.
19. Jinek M, East A, Cheng A, Lin S, Ma E, et al. (2013) RNA-programmed genome editing in human cells. Elife 2: e00471.
20. Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, et al. (2013) Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell 152: 1173-1183.
21. Maeder ML, Linder SJ, Cascio VM, Fu Y, Ho QH, et al. (2013) CRISPR RNA-guided activation of endogenous human genes. Nat Methods 10: 977-979.
22. Perez-Pinera P, Kocak DD, Vockley CM, Adler AF, Kabadi AM, et al. (2013) RNA-guided gene activation by CRISPR-Cas9-based transcription factors. Nat Methods 10: 973-976.
23. Mali P, Aach J, Stranges PB, Esvelt KM, Moosburner M, et al. (2013) CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. Nat Biotechnol 31: 833-838.
24. Cheng AW, Wang H, Yang H, Shi L, Katz Y, et al. (2013) Multiplexed activation of endogenous genes by CRISPR-on, an RNA-guided transcriptional activator system. Cell Res 23: 1163-1171.
25. Hilton IB, D'Ippolito AM, Vockley CM, Thakore PI, Crawford GE, et al. (2015) Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. Nat Biotechnol 33: 510-517.
26. Ledford H (2016) CRISPR: Gene editing is just the beginning. Nature 531: 156-159.
27. Braff JL, Yaung SJ, Esvelt KM, Church GM (2016) Characterization of Cas9-Guide RNA Orthologs. Cold Spring Harb Protoc pdb top086793.