CRISPR-CAS EFFECTOR POLYPEPTIDES AND METHODS OF USE THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/952,909, filed December 23, 2019, which application is incorporated herein by reference in its entirety.

INTRODUCTION

[0002] CRISPR-Cas systems include Cas proteins, which are involved in acquisition, targeting and cleavage of foreign DNA or RNA, and a guide RNA(s), which includes a segment that binds Cas proteins and a segment that binds to a target nucleic acid. For example, Class 2 CRISPR-Cas systems comprise a single Cas protein bound to a guide RNA, where the Cas protein binds to and cleaves a targeted nucleic acid. The programmable nature of these systems has facilitated their use as a versatile technology for use in modification of target nucleic acid.

SUMMARY

[0003] The present disclosure provides RNA-guided CRISPR-Cas effector proteins, nucleic acids encoding same, and compositions comprising same. The present disclosure provides ribonucleoprotein complexes comprising: an RNA-guided CRISPR-Cas effector protein of the present disclosure; and a guide RNA. The present disclosure provides methods of modifying a target nucleic acid, using an RNA-guided CRISPR-Cas effector protein of the present disclosure and a guide RNA.

[0003a] In one aspect, the present disclosure refers to a composition comprising:

a) a Cas12L polypeptide, or a nucleic acid molecule encoding the Cas12L polypeptide; and

b) a Cas12L guide RNA, or one or more DNA molecules encoding the Cas12L guide RNA.

[0003b] In one aspect, the present disclosure refers to a Cas12L fusion polypeptide comprising: a Cas12L polypeptide fused to a heterologous polypeptide.

[0003c] In one aspect, the present disclosure refers to a nucleic acid comprising a nucleotide sequence encoding any one of the Cas12L fusion polypeptides disclosed herein.

[0003d] In one aspect, the present disclosure refers to a eukaryotic cell comprising one or more of:

a) a Cas12L polypeptide, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L polypeptide,

b) a Cas12L fusion polypeptide, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L fusion polypeptide, and

c) a Cas12L guide RNA, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L guide RNA.

[0003e] In one aspect, the present disclosure refers to a cell comprising a comprising a Cas12L fusion polypeptide, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L fusion polypeptide.

[0003f] In one aspect, the present disclosure refers to a method of modifying a target nucleic acid, the method comprising contacting the target nucleic acid with:

a) a Cas12L polypeptide; and

b) a Cas12L guide RNA comprising a guide sequence that hybridizes to a target sequence of the target nucleic acid,

wherein said contacting results in modification of the target nucleic acid by the Cas12L polypeptide.

[0003g] In one aspect, the present disclosure refers to a method of modulating transcription from a target DNA, modifying a target nucleic acid, or modifying a protein associated with a target nucleic acid, the method comprising contacting the target nucleic acid with:

a) a Cas12L fusion polypeptide comprising a Cas12L polypeptide fused to a heterologous polypeptide; and

b) a Cas12L guide RNA comprising a guide sequence that hybridizes to a target sequence of the target nucleic acid.

[0003h] In one aspect, the present disclosure refers to a system comprising:

a) a Cas12L polypeptide and a Cas12L guide RNA;

b) a Cas12L polypeptide, a Cas12L guide RNA, and a DNA donor template;

c) a Cas12L fusion polypeptide and a Cas12L guide RNA;

d) a Cas12L fusion polypeptide, a Cas12L guide RNA, and a DNA donor template;

e) an mRNA encoding a Cas12L polypeptide, and a Cas12L guide RNA;

f) an mRNA encoding a Cas12L polypeptide; a Cas12L guide RNA, and a DNA donor template;

g) an mRNA encoding a Cas12L fusion polypeptide, and a Cas12L guide RNA;

h) an mRNA encoding a Cas12L fusion polypeptide, a Cas12L guide RNA, and a DNA donor template;

i) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a Cas12L polypeptide; and ii) a nucleotide sequence encoding a Cas12L guide RNA;

j) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a Cas12L polypeptide; ii) a nucleotide sequence encoding a Cas12L guide RNA; and iii) a DNA donor template;
k) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a Cas12L fusion polypeptide; and ii) a nucleotide sequence encoding a Cas12L guide RNA; and
l) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a Cas12L fusion polypeptide; ii) a nucleotide sequence encoding a Cas12L guide RNA; and a DNA donor template.

[0003i] In one aspect, the present disclosure refers to a kit comprising the Cas12L system disclosed herein.

[0003j] In one aspect, the present disclosure refers to an implantable device comprising the Cas12L system disclosed herein.

[0003k]In one aspect, the present disclosure refers to a method of detecting a target DNA in a sample, the method comprising:

(a) contacting the sample with:

(i) a Cas12L polypeptide;

(ii) a guide RNA comprising: a region that binds to the Cas12L polypeptide, and a guide sequence that hybridizes with the target DNA; and

(iii) a detector nucleic acid that is single stranded and does not hybridize with the guide sequence of the guide RNA; and

(b) measuring a detectable signal produced by cleavage of the single stranded detector nucleic acid by the Cas12L polypeptide, thereby detecting the target DNA.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] **FIG. 1** presents a maximum Likelihood phylogenetic tree of the Cas12 superfamily, produced from a multiple sequence alignment with 1000 iterations. Cas12L forms a distinct clade separate from other Cas12 proteins.

[0005] **FIG. 2A-2Z, 2AA-2ZZ, and 2AAA-2FFF** provide amino acid sequences of Cas12L polypeptides.

[0006] **FIG. 3A-3B** provide the locations of active site residues of the RuvC-I, RuvC-II, and RuvC-III domains of Cas12L polypeptides.

[0007] **FIG. 4** depicts CRISPR locus architecture with active sites annotated.

CLAIMS

What is claimed is:

1. A composition comprising:
a) a Cas12L polypeptide, or a nucleic acid molecule encoding the Cas12L polypeptide; and
b) a Cas12L guide RNA, or one or more DNA molecules encoding the Cas12L guide RNA.

2. The composition of claim 1, wherein the Cas12L polypeptide comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence depicted in any one of FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.

3. The composition of claim 1 or claim 2, wherein the Cas12L guide RNA comprises a nucleotide sequence having 80%, 90%, 95%, 98%, 99%, or 100%, nucleotide sequence identity with any one of the crRNA sequences depicted in FIG. 7, FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.

4. The composition of claim 1 or claim 2, wherein the Cas12L polypeptide is fused to a nuclear localization signal (NLS).

5. The composition of any one of claims 1-4, wherein:
a) the composition comprises a lipid;
b) components (a) and (b) are within a liposome; or
c) components (a) and (b) are within a particle.

6. The composition of any one of claims 1-5, wherein the Cas12L polypeptide is a nickase that can cleave only one strand of a double-stranded target nucleic acid molecule.

7. The composition of any one of claims 1-5, wherein the Cas12L polypeptide is a catalytically inactive Cas12L polypeptide (dCas12L).

8. The composition of any one of claims 1-7, wherein the Cas12L polypeptide has a length of from 700 amino acids to 800 amino acids.

9. The composition of any one of claims 1-8, further comprising a DNA donor template.

10. A Cas12L fusion polypeptide comprising: a Cas12L polypeptide fused to a heterologous polypeptide.

11. The Cas12L fusion polypeptide of Claim 10, wherein the Cas12L polypeptide comprises an amino acid sequence having 50% or more identity to the amino acid sequence depicted in any one of FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.

12. The Cas12L fusion polypeptide of claim 10 or claim 11, wherein the Cas12L polypeptide is a nickase that can cleave only one strand of a double-stranded target nucleic acid molecule, or wherein the Cas12L polypeptide is a catalytically inactive Cas12L polypeptide (dCas12L).

13. The Cas12L fusion polypeptide of any one of claims 10-12, comprising a nuclear localization signal (NLS).

14. The Cas12L fusion polypeptide of any one of claims 10-13, wherein the heterologous polypeptide exhibits enzymatic activity.

15. The Cas12L fusion polypeptide of claim 14, wherein the heterologous polypeptide exhibits one or more enzymatic activities selected from: reverse transcriptase activity, nuclease activity, methyltransferase activity, demethylase activity, deamination activity, depurination activity, integrase activity, transposase activity, and recombinase activity.

16. The Cas12L fusion polypeptide of any one of claims 10-13, wherein the heterologous polypeptide is a protein that increases or decreases transcription.

17. A nucleic acid comprising a nucleotide sequence encoding the Cas12L fusion polypeptide of any one of claims 10-16.

18. A eukaryotic cell comprising one or more of:
a) a Cas12L polypeptide, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L polypeptide,
b) a Cas12L fusion polypeptide, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L fusion polypeptide, and
c) a Cas12L guide RNA, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L guide RNA.

19. A cell comprising a comprising a Cas12L fusion polypeptide, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L fusion polypeptide.

20. A method of modifying a target nucleic acid, the method comprising contacting the target nucleic acid with:
a) a Cas12L polypeptide; and
b) a Cas12L guide RNA comprising a guide sequence that hybridizes to a target sequence of the target nucleic acid,
wherein said contacting results in modification of the target nucleic acid by the Cas12L polypeptide.

21. A method of modulating transcription from a target DNA, modifying a target nucleic acid, or modifying a protein associated with a target nucleic acid, the method comprising contacting the target nucleic acid with:
a) a Cas12L fusion polypeptide comprising a Cas12L polypeptide fused to a heterologous polypeptide; and
b) a Cas12L guide RNA comprising a guide sequence that hybridizes to a target sequence of the target nucleic acid.

22. A system comprising:
a) a Cas12L polypeptide and a Cas12L guide RNA;
b) a Cas12L polypeptide, a Cas12L guide RNA, and a DNA donor template;
c) a Cas12L fusion polypeptide and a Cas12L guide RNA;
d) a Cas12L fusion polypeptide, a Cas12L guide RNA, and a DNA donor template;
e) an mRNA encoding a Cas12L polypeptide, and a Cas12L guide RNA;
f) an mRNA encoding a Cas12L polypeptide; a Cas12L guide RNA, and a DNA donor template;
g) an mRNA encoding a Cas12L fusion polypeptide, and a Cas12L guide RNA;
h) an mRNA encoding a Cas12L fusion polypeptide, a Cas12L guide RNA, and a DNA donor template;
i) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a Cas12L polypeptide; and ii) a nucleotide sequence encoding a Cas12L guide RNA;
j) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a Cas12L polypeptide; ii) a nucleotide sequence encoding a Cas12L guide RNA; and iii) a DNA donor template;
k) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a Cas12L fusion polypeptide; and ii) a nucleotide sequence encoding a Cas12L guide RNA; and

l) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a Cas12L fusion polypeptide; ii) a nucleotide sequence encoding a Cas12L guide RNA; and a DNA donor template.

23. A kit comprising the Cas12L system of claim 22.

24. An implantable device comprising the Cas12L system of claim 22.

25. A method of detecting a target DNA in a sample, the method comprising:

(a) contacting the sample with:

(i) a Cas12L polypeptide;

(ii) a guide RNA comprising: a region that binds to the Cas12L polypeptide, and a guide sequence that hybridizes with the target DNA; and

(iii) a detector nucleic acid that is single stranded and does not hybridize with the guide sequence of the guide RNA; and

(b) measuring a detectable signal produced by cleavage of the single stranded detector nucleic acid by the Cas12L polypeptide, thereby detecting the target DNA.

26. The method of claim 25, wherein the target DNA is bacterial DNA, viral DNA, or DNA from a human cell.

27. The method of claim 25, further comprising amplifying the target DNA in the sample by loop-mediated isothermal amplification (LAMP), helicase-dependent amplification (HDA), recombinase polymerase amplification (RPA), strand displacement amplification (SDA), nucleic acid sequence-based amplification (NASBA), transcription mediated amplification (TMA), nicking enzyme amplification reaction (NEAR), rolling circle amplification (RCA), multiple displacement amplification (MDA), Ramification (RAM), circular helicase-dependent amplification (cHDA), single primer isothermal amplification (SPIA), signal mediated amplification of RNA technology (SMART), self-sustained sequence replication (3SR), genome exponential amplification reaction (GEAR), or isothermal multiple displacement amplification (IMDA).

28. The method of any one of claims 25-27, wherein target DNA in the sample is present at a concentration of less than 10 aM.