general method for engineering programmable RNA effector proteins. Similar to CRISPR/Cas-based systems, CIRTS is a ribonucleoprotein complex that uses Watson-Crick-Franklin base pair interactions to deliver protein cargo site-selectively in the transcriptome. The inventors show they can easily engineer CIRTS that deliver a range or regulatory proteins to transcripts, including nucleases for degradation, deadenylation regulatory machinery for degradation, or translational activation machinery for enhanced protein production. However, CIRTS are up to 5-fold smaller than the smallest current CRISPR/Cas systems and can be engineered entirely from human parts.

[0007a] In one aspect, provided herein is a RNA regulatory system comprising at least one of each: i) at least one RNA hairpin binding domain; ii) at least one RNA targeting molecule comprising a RNA targeting region and at least one hairpin structure, wherein the hairpin structure of the RNA targeting molecule specifically binds to i; and iii) at least one RNA regulatory domain wherein the at least one RNA hairpin binding domain and/or the at least one hairpin structure are human or are human-derived; and wherein: (a)the at least one RNA hairpin binding domain and the at least one RNA regulatory domain are present in a single fusion protein, or (b) the at least one RNA regulatory domain is covalently linked to a first dimerization domain and the at least one RNA hairpin binding domain is covalently linked to a second dimerization domain, wherein the first and second dimerization domains are capable of dimerizing to form a non-covalent or covalent linkage.

[0007b] In one aspect, provided herein is a system comprising one or more nucleic acids including: i) a nucleic acid that encodes a polypeptide comprising a RNA hairpin binding domain; ii) a nucleic acid that is or encodes a RNA targeting molecule comprising a RNA targeting region and at least one hairpin structure, wherein the at least one hairpin structure of the RNA targeting molecule specifically binds to i), and iii) a nucleic acid that encodes a polypeptide comprising a RNA regulatory domain; wherein the RNA hairpin binding domain and/or the hairpin structure are human or are human-derived, wherein: (a) a single nucleic acid comprises the nucleic acid that encodes the polypeptide comprising the RNA hairpin binding domain and the nucleic acid that encodes the polypeptide comprising the RNA regulatory domain, and the RNA hairpin binding domain and the RNA regulatory domain are present in a single fusion protein, or (b) the polypeptide that comprises the RNA regulatory domain further comprises a first dimerization domain that is covalently linked to the RNA regulatory domain, and the polypeptide that comprises the RNA hairpin binding domain further comprises a second dimerization domain that is covalently linked to the RNA hairpin binding

domain, wherein the first and second dimerization domains are capable of dimerizing to form a non-covalent or covalent linkage.

[0007c] In one aspect, provided herein is a fusion protein comprising: a) a RNA hairpin binding domain and a RNA regulatory domain; or b) a RNA regulatory domain and a first dimerization domain; or c) a RNA hairpin binding domain and a second dimerization domain, wherein the RNA hairpin binding domain and/or the hairpin structure are human or are human-derived.

[0007d] In one aspect, provided herein is a nucleic acid encoding the fusion protein as disclosed herein.

[0007e] In one aspect, provided herein is a delivery vehicle comprising the system as disclosed herein, or the fusion protein as disclosed herein, and optionally wherein the delivery vehicle comprises liposome(s), particle(s), exosome(s), microvesicle(s), a gene-gun or one or more nucleic acid vector(s).

[0007f] In one aspect, provided herein is a composition comprising the system, the fusion protein, or the delivery vehicle as disclosed herein.

[0007g] In one aspect, provided herein is a cell comprising the system, the fusion protein, the delivery vehicle, or the composition as disclosed herein.

[0007g] In one aspect, provided herein is a method of modulating at least one target RNA comprising contacting the target RNA with the system, the fusion protein, the delivery vehicle, or the composition as disclosed herein, wherein the method is not an *in vivo* method.

[0007h] In one aspect, provided herein is a kit comprising the system, the fusion protein, the delivery vehicle, or the composition as disclosed herein.

[0007i] In one aspect, provided herein is the system, the fusion protein, the delivery vehicle, or the composition for use in therapy.

[0007j] In one aspect, provided herein is a RNA regulatory system comprising: i) at least one RNA hairpin binding domain; ii) at least one RNA targeting molecule comprising a RNA targeting region and at least one hairpin structure, wherein the hairpin structure of the RNA targeting molecule specifically binds to i); and iii) at least one RNA regulatory domain,wherein the at least one RNA hairpin binding domain and/or the at least one hairpin structure are human or are human-derived; and wherein the RNA regulatory system further comprises a stabilizer polypeptide.

[0008] Examples of the disclosure relate to a RNA regulatory system or method comprising at least one of each: i) a RNA hairpin binding domain; ii) a RNA targeting molecule comprising a RNA targeting region and at least one hairpin structure, wherein the hairpin structure of the RNA targeting molecule specifically binds to i; and iii) a RNA regulatory domain. In some examples, the following are included: i) and ii), i) and iii), ii) and iii), or i), ii), and iii). Any example disclosed herein can contain any of these combinations.

[0009] Further examples relate to a vector system comprising one or more nucleic acid vectors comprising a nucleotide encoding: i) a RNA hairpin binding domain; ii) a RNA targeting molecule comprising a RNA targeting region and at least one hairpin structure, wherein the hairpin structure of the RNA targeting molecule specifically binds to i), and iii) a RNA regulatory domain.

[0010] Further examples relate to a fusion protein comprising a RNA hairpin binding protein and a RNA regulatory domain and nucleic acids encoding such fusion proteins.

[0011] Further aspects relate to a conjugate comprising a RNA regulatory domain operably linked to a RNA targeting molecule, wherein the RNA targeting molecule comprises a RNA targeting region and at least one hairpin structure. In some examples, the RNA regulatory domain and the RNA targeting molecule are operably linked through a peptide bond. In some examples, the polypeptide further comprises one or more linkers. In some examples, the RNA regulatory domain and the RNA targeting molecule are operably linked through non-covalent interactions. In some examples, the RNA regulatory domain is covalently linked to a first dimerization domain and the RNA targeting molecule is covalently linked to a second

CLAIMS

1. A RNA regulatory system comprising:

i) at least one RNA hairpin binding domain;

ii) at least one RNA targeting molecule comprising a RNA targeting region and at least one hairpin structure, wherein the hairpin structure of the RNA targeting molecule specifically binds to i); and

iii) at least one RNA regulatory domain,

wherein the at least one RNA hairpin binding domain and/or the at least one hairpin structure are human or are human-derived; and

wherein:

(a) the at least one RNA hairpin binding domain and the at least one RNA regulatory domain are present in a single fusion protein, or

(b) the at least one RNA regulatory domain is covalently linked to a first dimerization domain and the at least one RNA hairpin binding domain is covalently linked to a second dimerization domain, wherein the first and second dimerization domains are capable of dimerizing to form a non-covalent or covalent linkage.

2. The system of claim 1, wherein the dimerization is inducible,

optionally wherein the dimerization comprises ligand-induced dimerization, and

further optionally wherein:

- one of the first or second dimerization domain comprises PYR/PYR1-like (PYL1), the other of the first or second dimerization domain comprises ABA insensitive 1 (ABI1), and the ligand comprises abscisis acid (ABA) or derivatives or fragments thereof; or
- the first and/or second dimerization domain comprises FKBP12 and the ligand comprises FK1012 or derivatives or fragments thereof; or
- one of the first or second dimerization domains comprises FK506 binding protein (FKBP), the other of the first or second dimerization domain comprises FKBP-Rap binding domain of mammalian target of Rap mTOR (Frb), and the ligand comprises rapamycin (Rap) or derivatives or fragments thereof.

3. The system of claim 1 or claim 2, wherein the system further comprises a stabilizer polypeptide.

4. A RNA regulatory system comprising:

i) at least one RNA hairpin binding domain;

ii) at least one RNA targeting molecule comprising a RNA targeting region and at least one hairpin structure, wherein the hairpin structure of the RNA targeting molecule specifically binds to i); and

iii) at least one RNA regulatory domain,

wherein the at least one RNA hairpin binding domain and/or the at least one hairpin structure are human or are human-derived; and

wherein the RNA regulatory system further comprises a stabilizer polypeptide.

5. The system of claim 4, wherein:

A) the at least one RNA hairpin binding domain and the at least one RNA regulatory domain are present in a single fusion protein, or

B) the at least one RNA regualtory domain is covalently linked to a first dimerization domain and the at least one RNA hairpin binding domain is covalently linked to a second dimerization domain, wherein the first and second dimerization domains are capable of dimerizing to form a non-covaleny or covalent linkage, optionally wherein the dimerization is inducible, further optionally wherein the dimerization comprises ligand-induced dimerization, and further optionally wherein:

- one of the first or second dimerization domain comprises PYR/PYR1-like (PYL1), the other of the first or second dimerization domain comprises ABA insensitive 1 (ABI1), and the ligand comprises abscisis acid (ABA) or derivatives or fragments thereof; or
- the first and/or second dimerization domain comprises FKBP12 and the ligand comprises FK1012 or derivatives or fragments thereof; or
- one of the first or second dimerization domains comprises FK506 binding protein (FKBP), the other of the first or second dimerization domain comprises FKBP-Rap binding domain of mammalian target of Rap mTOR (Frb), and the ligand comprises rapamycin (Rap) or derivatives or fragments thereof

6. The RNA regulatory system of any one of claims 3-5, wherein the stabilizer polypeptide comprises a cationic polypeptide that binds non-specifically to nucleic acids,

optionally wherein the stabilizer polypeptide comprises HBEGF, beta-defensin, or variants or portions thereof, and

further optionally wherein the stabilizer polypeptide is human-derived.

7. The system of any one of claims 1-6, wherein:

A) ii) comprises at least two hairpin structures; and/or

B) ii) comprises one or more modified nucleotides; and/or

C) the total size of the system is less than 150 kDa; and/or

D) i) comprises U1A, SLBP, or variants thereof; and/or

E) ii) comprises a TAR hairpin scaffold of SEQ ID NO:1; and/or

F) ii) comprises a SLBP hairpin scaffold of SEQ ID NO:2; and/or

G) ii) comprises a linker, optionally wherein the linker is at least 5 amino acids; and/or

H) the RNA targeting region comprises at least 12 nucleotides; and/or

I) iii) comprises a nuclease, methylase, demethylase, translational activator, translational repressor, single-stranded RNA cleavage activity, double-stranded RNA cleavage activity, or RNA binding activity; and/or

J) iii) comprises a Pin nuclease domain or a m6A reader protein or portion thereof, optionally wherein iii) comprises YTHDF1, YTHDF2, or ADAR; and/or

K) the RNA targeting region of ii) hybridizes to a target RNA in a prokaryotic or eukaryotic cell; and/or

L) i) and/or iii) comprises one or more nuclear localization signals (NLS)s and/or

M) the system comprises at least two of each i), ii), and iii); and/or

N) the RNA regulatory domain cleaves RNA, promotes RNA translation, inhibits RNA translation, or modifies the base sequence of RNA.

8. A system comprising one or more nucleic acids including: i) a nucleic acid that encodes a polypeptide comprising a RNA hairpin binding domain; ii) a nucleic acid that is or encodes a RNA targeting molecule comprising a RNA targeting region and at least one hairpin structure, wherein the at least one hairpin structure of the RNA targeting molecule specifically binds to i), and iii) a nucleic acid that encodes a polypeptide comprising a RNA regulatory domain; wherein the RNA hairpin binding domain and/or the hairpin structure are human or are human-derived,

wherein:

(a) a single nucleic acid comprises the nucleic acid that encodes the polypeptide comprising the RNA hairpin binding domain and the nucleic acid that encodes the polypeptide comprising the RNA regulatory domain, and the RNA hairpin binding domain and the RNA regulatory domain are present in a single fusion protein, or

(b) the polypeptide that comprises the RNA regulatory domain further comprises a first dimerization domain that is covalently linked to the RNA regulatory domain, and the polypeptide that comprises the RNA hairpin binding domain further comprises a second dimerization domain that is covalently linked to the RNA hairpin binding domain, wherein the first and second dimerization domains are capable of dimerizing to form a non-covalent or covalent linkage.

9. The system of claim 8, wherein:

A) the system further comprises a regulatory element operably linked to i), ii), and/or iii); and/or

B) the one or more nucleic acids are optimized for expression in an eukaryotic cell, optionally wherein the expression is constitutive or conditional; and/or

C) i), ii), and iii) are on a single nucleic acid; and/or

D) wherein:

a) one or more of the nucleic acids are viral vectors, optionally wherein the one or more nucleic acids comprise one or more retroviral, lentiviral, adenoviral, adeno-associated or herpes simplex viral vectors; or

b) one or more of the nucleic acids are non-viral vectors.

10. A fusion protein comprising:

a) a RNA hairpin binding domain and a RNA regulatory domain; or

b) a RNA regulatory domain and a first dimerization domain; or

c) a RNA hairpin binding domain and a second dimerization domain,

wherein the RNA hairpin binding domain and/or the hairpin structure are human or are human-derived.

11. The fusion protein of claim 10(b) or 10(c), wherein dimerization of the first and/or second dimerization domain is inducible, optionally wherein the dimerization comprises ligand-induced dimerization, and further optionally wherein:

i) the first and second dimerization domains are selected from PYL1 and ABI1 and the ligand comprises ABA or derivatives or fragments thereof; or

ii) the first and/or second dimerization domain comprises FKBP12 and the ligand comprises FK1012 or derivatives or fragments thereof; or

iii) first and second dimerization domains are selected from FKBP and Frb, and the ligand comprises rapamycin or derivatives or fragments thereof.

12. The fusion protein of any one of claim 10 or 11, wherein:

A) the RNA hairpin binding domain and/or RNA regulatory domain are human-derived; and/or

B) the fusion protein is less than 150 kDa; and/or

C) the RNA hairpin binding domain comprises U1A, SLBP, or variants or fragments thereof and/or

D) the RNA regulatory domain comprises a nuclease, methylase, demethylase, translational activator, translational repressor, single-stranded RNA cleavage activity, double-stranded RNA cleavage activity, or RNA binding activity; and/or

E) the RNA regulatory domain comprises a Pin nuclease domain or a m6A reader protein or portion thereof, optionally wherein the RNA regulatory domain comprises YTHDF1 or YTHDF2; and/or

F) the fusion protein further comprises one or more nuclear localization signals (NLS)s; and/or

G) the RNA regulatory domain cleaves RNA, promotes RNA translation, inhibits RNA translation, or modifies the base sequence of RNA.

13. A nucleic acid encoding the fusion protein of any one of claims 10-12.

14. A delivery vehicle comprising the system of any one of claims 1-9 or the fusion protein of any one of claims 10-12, and optionally wherein the delivery vehicle comprises liposome(s), particle(s), exosome(s), microvesicle(s), a gene-gun or one or more nucleic acid vector(s).

15. A composition comprising the system of any one of claims 1-9, the fusion protein of any one of claims 10-12, or the delivery vehicle of claim 14.

16. A cell comprising the system of any one of claims 1-9, the fusion protein of any one of claims 10-12, the delivery vehicle of claim 14, or the composition of claim 15.

17. A method of modulating at least one target RNA comprising contacting the target RNA with the system of any one of claims 1-9, the fusion protein of any one of claims 10-12, the delivery vehicle of claim 14, or the composition of claim 15, wherein the method is not an *in vivo* method.

18. The method of claim 17, wherein:

a) modulating the at least one target RNA comprises cleaving, demethylating, methylating, activating translation, repressing translation, promoting degradation, and/or binding to the RNA; and/or

b) the target RNA is in a prokaryotic or eukaryotic cell, and optionally wherein:

i) the target RNA is in a human cell; and/or

ii) the target RNA is in vitro.

19. A kit comprising the system of any one of claims 1-9, the fusion protein of any one of claims 10-12, the delivery vehicle of claim 14, or the composition of claim 15.

20. The system of any one of claims 1-9, the fusion protein of any one of claims 10-12, the delivery vehicle of claim 14, or the composition of claim 15 for use in therapy.