

2'-FLUORO RNA ACTIVATORS FOR ENHANCED ACTIVATION OF CSM6 IN RNA DETECTION ASSAYS

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OTHER INFORMATION

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PATENT STATUS

Patent Pending

BRIEF DESCRIPTION

Csm6 constitutes a family of enzymes that are activated by cyclic oligoadenylates (cA(n)) or linear oligoadenylates with a 2',3'-cyclic phosphate termini (A(n)>P). Cleavage of a nucleic acid sequence by an RNase to generate a linear oligoadenylate with exactly 4 or 6 A's and the 2',3'-cyclic phosphate terminus (A4>P or A6>P) leads to activation of Csm6/Csx1 for cleavage of a fluorescent RNA reporter. The linear A4 or A6 can be incorporated into an RNA sequence (e.g. A4-U6 or A6-U5) such that activation of Csm6 only occurs upon removal of the U-containing sequence by Cas13a, a programmable RNA-guided RNase that preferentially cleaves the phosphodiester bond that is 5' to U's and generates products with 2',3'-cyclic phosphates. Csm6 is normally inactivated through self-cleavage of its activator, leading to low sensitivity when coupled with a Cas13-based RNA detection system or a Cas13-Csm6 feed-forward detection system.

In this invention, the 2'-hydroxyl of the ribose in the second A in the linear A4 or the third A in the linear A6 is replaced with a 2'-fluorine (fA). This single 2'-fluoro modified RNA oligonucleotide (A-fA-AA>P or AA-fA-AAA>P) would bind and activate Csm6/Csx1 with fast kinetics and prevent degradation of the linear oligoadenylate by Csm6/Csx1. This single 2'-fluoro-modified polyA activator could be followed by any sequence to couple activation of Csm6 to a second enzyme. The purpose of this invention is to generate sustained activation of Csm6, when coupled with a Cas13 RNA detection system. In one iteration of this invention, the modified activator is followed by a linear chain of U's, and is thus cleavable by Cas13 upon Cas13's activation by a complementary sequence of RNA. Other nucleotides (e.g. C, A) or 2'-deoxy modifications can also be included 3' to the first U to restrict the cleavage of Cas13a to the precise site that is required to release the single 2'-fluoro modified An>P (e.g. A-fA-AAUCCCCC...). This activator leads to increased sensitivity and kinetics in RNA detection when coupled with Cas13. In another iteration of this invention, the modified activator is followed by a linear chain of C's (Cn). This substrate can be acted upon by a pre-activated Csm6 (e.g. by Cas13) to produce A-fA-AA>P or AA-fA-AAA>P, which initiates a sustained feed-forward loop and prevents self-degradation of the activator by Csm6. Restricting the cleavage site of this activator by addition of chemical modifications (such as 2'-deoxy) on positions other than the cleavage site leads to a precise cut by Csm6.

This activator can be combined with the previous iteration to generate even higher sensitivity and kinetics in RNA detection than the previous iteration alone. Cleavage of a fluorescent and colorimetric RNA reporter by the highly activated Csm6 in either iteration would then generate a detectable signal. In addition, nucleotides with modified bases that are not recognized by Csm6 or Cas13 may also be used in the cleavable "tail" of the activators to avoid competition with the RNA reporter or other activators in the system. Overall, the purpose of this invention is to enable elevated activation and kinetics of Csm6 when coupled with a Cas13 RNA detection system or a feed-forward reaction with Csm6 and Cas13. This could be used in low-copy detection of any type of single-stranded RNA, including viral RNA genomes, viral RNA transcripts, and cellular RNA transcripts. In addition, these activators could also be used with the related family of enzymes known as Csx1.

SUGGESTED USES

This technology could be used for in vitro detection of any type of ssRNA (e.g. viral genomic RNA or RNA transcripts, or mRNA from cellular extracts or metagenomic samples).

ADVANTAGES

RELATED MATERIALS

ADDITIONAL TECHNOLOGIES BY THESE INVENTORS

- [Compositions and Methods for Genome Editing in Plants](#)
- [Compression of Genetic Information in Multiple Reading Frames](#)
- [Composition and Methods of a Nuclease Chain Reaction for Nucleic Acid Detection](#)

