

SYSTEM FOR REGULATING GENE EXPRESSION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/894,611, filed on August 30, 2019, U.S. Provisional Application No. 62/904,635, filed on September 23, 2019, and U.S. Provisional Application No. 63/043,504, filed June 24, 2020, the contents of each of which are incorporated herein by reference in their entirety.

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[0002] This invention was made with government support under EB013584 awarded by the National Institutes of Health. The government has certain rights in the invention.

Background

[0003] Nucleic acid based constructs for modulating expression of genes can be improved by increasing sensitivity and reducing leakiness.

Summary

[0004] The present disclosure recognizes a discovery of nucleic acid constructs related to regulatable gene product expression. In some embodimentexamples, the present disclosure provides compositions and methods for the regulation of gene expression using nucleic acid constructs. In some embodimentexamples, the present disclosure recognizes the utility of alternative splicing in regulation of gene expression in a nucleic acid construct. In some embodimentexamples, the present disclosure recognizes the utility of regulating gene expression utilizing ligand-binding aptamers.

[0004a] In one aspect, the present disclosure refers to a system for modulating gene expression, comprising a polyA aptamer polynucleotide that comprises in a 5' to 3' direction: a) a 5' splice donor site; b) an engineered intron; c) a first 3' splice acceptor site; d) a polyA switch comprising two or more ligand-binding aptamers with one or more ligand binding pockets, and at least one polyA cleavage signal therein; e) a second 3' splice acceptor site; and f) a nucleic acid sequence encoding an expressible polypeptide.

[0004b] In another aspect, the present disclosure refers to a vector for delivery of the system as disclosed herein.

[0004c] In another aspect, the present disclosure refers to the use of a composition comprising a system for manufacture of a medicament for modulating expression of a gene product in a cell, the system comprising in a 5' to 3' direction: a) a 5' splice donor site; b) an engineered intron; c) a first 3' splice acceptor site; d) a polyA switch comprising two or more ligand-binding aptamers with one or more ligand binding pockets, and at least one polyA cleavage signal therein; and e) a second 3' splice acceptor site.

[0004d] In another aspect, the present disclosure refers to a nucleic acid molecule encoding the poly A aptamer polynucleotide comprising in a 5' to 3' direction: a) a 5' splice donor site; b) an engineered intron; c) a first 3' splice acceptor site; d) a polyA switch comprising two or more ligand-binding aptamers with one or more ligand binding pockets, and at least one polyA cleavage signal therein; e) a second 3' splice acceptor site; and f) a nucleic acid sequence encoding an expressible polypeptide.

[0004e] In another aspect, the present disclosure refers to a vector for delivery of the nucleic acid as disclosed herein.

[0005] In some embodimentexamples, the present disclosure provides a system for modulating gene expression, comprising a polyA aptamer polynucleotide that comprises in a 5' to 3' direction: a 5' splice donor site; an engineered intron; a first 3' splice acceptor site; a polyA switch comprising two or more ligand-binding aptamers with one or more ligand binding pockets, and at least one polyA cleavage signal therein; a second 3' splice acceptor site; and a nucleic acid sequence encoding an expressible polypeptide.

[0006] In some embodimentexamples, a polyA aptamer polynucleotide of the present disclosure comprises two ligand-binding aptamers. In some embodimentexamples, a polyA aptamer

polynucleotide comprises three ligand-binding aptamers. In some [embodimentexamples](#), a polyA aptamer polynucleotide comprises a polyA switch comprising a three way junction. In some [embodimentexamples](#), a three way junction comprises a junction of one or more RNA double stranded stems. In some [embodimentexample](#)s, portions of a three way junction are single stranded. In some [embodimentexamples](#), a RNA double stranded stem comprises a ligand-binding aptamer. In some [embodimentexample](#)s, a nucleic acid sequence encoding an expressible polypeptide comprises a 5'UTR.

[0007] In some [embodimentexamples](#), the present disclosure provides a method for modulating expression of a gene product in a cell. The method comprises the steps of: introducing into the cell a system comprising in a 5' to 3' direction: a 5' splice donor site; an engineered intron; a first 3' splice acceptor site; a polyA switch comprising two or more ligand-binding aptamers with one or more ligand binding pockets, and at least one polyA cleavage signal therein; a second 3' splice acceptor site. In some [embodimentexample](#)s a gene product expressed by the methods described herein is exogenous to the cell. In some [embodimentexamples](#), a gene product expressed by the methods described herein is endogenous to the cell. In some [embodimentexamples](#), a method provided by the present disclosure occurs in one or more cells of an individual, the ligand is glucose, the individual has diabetes, pre-diabetes, or complications from diabetes, and/or the expressible polynucleotide is insulin. In some [embodimentexamples](#), a method provided by the present disclosure occurs in one or more cells of an individual, the expressible polynucleotide is a therapeutic gene product such as human growth hormone, coagulation factor X, or dystrophin. In some [embodimentexamples](#), a method provided by the present disclosure occurs in one or more cells of an individual, the ligand is the gene product of a cancer biomarker, and the expressible polynucleotide is a suicide gene. In some [embodimentexample](#)s, a method provided by the present disclosure occurs in an individual, the expressible polynucleotide is a reporter gene, and the location and/or intensity of the expression of the reporter gene provides information about spatial distribution, temporal fluctuation, or both, of a ligand in one or more cells of the individual. In some [embodimentexamples](#), a method provided by the present disclosure occurs in an individual, tissue, or cell, wherein the expressible polynucleotide encodes a detectable gene product, and wherein the respective individual, tissue, or cell is imaged.

Claims

We claim:

1. A system for modulating gene expression, comprising a polyA aptamer polynucleotide that comprises in a 5' to 3' direction:
 - a) a 5' splice donor site;
 - b) an engineered intron;
 - c) a first 3' splice acceptor site;
 - d) a polyA switch comprising two or more ligand-binding aptamers with one or more ligand binding pockets, and at least one polyA cleavage signal therein;
 - e) a second 3' splice acceptor site; and
 - f) a nucleic acid sequence encoding an expressible polypeptide.
2. The system of claim 1, wherein the polyA switch comprises two ligand binding aptamers.
3. The system of claim 1, wherein the polyA switch comprises three ligand binding aptamers.
4. The system of claim 1, wherein the polyA switch comprises a three way junction.
5. The system of claim 4, wherein the three way junction comprises a junction of a first, a second, and a third double stranded RNA stem.
6. The system of claim 5, wherein the first double stranded RNA stem does not comprise a ligand binding aptamer.
7. The system of claim 5, wherein each of the first, second, and third double stranded RNA stems comprise a ligand binding aptamer.

8. The system of claim 5, wherein the three way junction comprises at least one single stranded region.

9. The system of claim 8, wherein the three way junction comprises a first, a second, and a third single stranded region.

10. The system of claim 9, wherein the first single stranded region is located between the first double stranded RNA stem and the second double stranded RNA stem.

11. The system of claim 9, wherein the second single stranded region is located between the second double stranded RNA stem and the third double stranded RNA stem.

12. The system of claim 9, wherein the third single stranded region is located between the third double stranded RNA stem and the first double stranded RNA stem of the first aptamer.

13. The system of any one of claims 1-12~~the preceding claims~~, wherein the first aptamer and the second aptamer, in a 5' to 3' orientation, are in the same orientation.

14. The system of any one of claims 3-13~~the preceding claims~~, wherein the third aptamer, in a 5' to 3' orientation, is in the opposite orientation relative to the first and second aptamers.

15. The system of claim 1, wherein one or more nucleotides of the ~~the~~ polyA cleavage signal are within the ~~3~~three way junction, the third double stranded RNA stem, the third single stranded region, or the first double stranded RNA stem.

16. The system of claim 15, wherein the third single stranded region comprises the first four bases of the polyA cleavage signal.

17. The system of claim 15, wherein the first double stranded RNA stem comprises the last two bases of the polyA cleavage signal.
18. The system of claim 15, wherein the first double stranded RNA stem comprises the entirety of the polyA cleavage signal.
19. The system of claim 3, wherein the double stranded RNA stem between the binding pocket of the third aptamer and the three way junction is between 10 and 15 base pairs in length.
20. The system of claim 10, wherein the first single stranded region comprises at least one base selected from C and A.
21. The system of claim 11, wherein the second single stranded region comprises at least one base selected from C and A.
22. The system of claim 5, wherein the sequence of the second double stranded RNA stem is SEQ ID NO.: 3.
23. The system of claim 5, wherein the sequence of the third double stranded RNA stem is SEQ ID NO.: 2.
24. The system of claim 5, wherein the sequence of the first double stranded RNA stem is SEQ ID NO.: 4.
25. The system of claim 5, wherein the sequence of the first double stranded RNA stem is SEQ ID NO.: 5.

26. The system of claim 1, wherein the nucleic acid sequence encoding the expressible polypeptide further comprises a 5'UTR.
27. The system of claim 26, wherein the 5'UTR further comprises a CAA repeat.
28. The system of claim 26, wherein the 5'UTR further comprises one or more 3' splice acceptor sites.
29. The system of claim 26, wherein the engineered 5'UTR has sequence SEQ ID NO.: 48.
30. The system of claim 1, further comprising a G-U rich region 5' of the nucleic acid sequence encoding the expressible polypeptide and 3' of the polyA cleavage signal.
31. The system of claim 29, where the 3'acceptor site is followed by a nucleic acid triplet sequence that modulates the strength of the alternative splicing.
32. The system of claim 31, wherein the nucleic acid triplet is 3' relative to the second 3'acceptor site in the 5'UTR and has a sequence selected from the following: TAG, TCT, TTC, TTG, TGA, TGC, TCC, ACA, AAC, ACC, AGC, AGG, CCT, and CCC.
33. The system of claim 1, further comprising a G rich region 5' of the nucleic acid sequence encoding the expressible polypeptide and 3' of the G-U rich region.
34. The system of claim 33, wherein the G rich-region comprises 4 MAZ sequence.
35. The system of claim 1, wherein the engineered intron has a sequence of between 100 and 200 bases in length.

36. The system of claim 1, wherein the engineered intron has sequence SEQ ID NO.: 1.

37. The system of claim 1, where the engineered intron is followed by a nucleic acid triplet sequence that modulates the strength of the intron splicing.

38. The system of claim 37, wherein the nucleic acid triplet sequence is a sequence selected from: TTT, TGA, TCT, TAC, CAC, and CAT.

39. The system of claim 1, wherein the system comprises a sequence selected from the group SEQ ID NO.:6 to SEQ ID NO.: 56.

40. The system of claim 39, wherein the system comprises a sequence selected from the group SEQ ID NO.:6 SEQ ID NO.:13; SEQ ID NO.:14; SEQ ID NO.:28; SEQ ID NO.:32; SEQ ID NO.:33; SEQ ID NO.:36; SEQ ID NO.:38; SEQ ID NO.:44; SEQ ID NO.:46; SEQ ID NO.: 50; NO.: 51; NO.: 52; NO.: 53; NO.: 54; NO.: 55; NO.: 56.

41. A vector for delivery of the system of claim 1.

42. The vector of claim 41, wherein the vector is a viral vector.

43. The vector of claim 42, wherein the vector is selected from an adenoviral vector, a lentiviral vector; an adeno-associated viral vector, a *poliovirus* vector, and a retrovirus vector.

44. ~~Use of a composition comprising a system for manufacture of a medicament~~A method for modulating expression of a gene product in a cell, the method comprising the steps of:
~~introducing into the cell a system comprising in a 5' to 3' direction:~~

a) a 5' splice donor site

b) an engineered intron

- c) a first 3' splice acceptor site
- d) a polyA switch comprising two or more ligand-binding aptamers with one or more ligand binding pockets, and at least one polyA cleavage signal therein; and
- e) a second 3' splice acceptor site.

45. The ~~use~~method of claim 44, wherein the gene product is exogenous to the cell.

46. The ~~use~~method of claim 45, wherein the system further comprises a nucleic acid sequence encoding the gene product immediately 3' of the splice site of e).

47. The ~~use~~method of claim 44, wherein the gene product is endogenous to the cell.

48. The ~~use~~method of claim 47, wherein the ~~method does not comprise administering the ligand is~~ not provided to the cell to inhibit expression of the endogenous gene product.

49. The ~~use~~method of claim 44, wherein the system further comprises a promoter 5' of the splice site of a).

50. The ~~use~~method of claim 49, wherein the promoter is a CMV promoter.

51. The ~~use~~method of any one of ~~claims 44-50~~the preceding claims, wherein the ~~cell is~~method occurs ~~in~~ one or more cells of an individual, the ligand is glucose, the individual has diabetes, pre-diabetes, or complications from diabetes, and/or the expressible polynucleotide is insulin.

52. The ~~use~~method of any one of ~~claim 44-50~~the preceding claims, wherein the ~~cell is~~method occurs ~~in~~ one or more cells of an individual, the ligand is the gene product of a cancer biomarker, and the expressible polynucleotide is a suicide gene.

53. The ~~use~~method of any one of ~~claims 44-52~~the preceding claims, wherein the ~~cell is~~method occurs in an individual, the expressible polynucleotide is a reporter gene, and the location and/or intensity of the expression of the reporter gene provides information about spatial distribution, temporal fluctuation, or both, of a ligand in one or more cells of the individual.

54. The ~~use~~method of any one of ~~claims 44-50~~the preceding claims, wherein the ~~cell is~~method occurs in an individual, tissue, or cell, ~~wherein~~ the expressible polynucleotide encodes a detectable gene product, and ~~wherein~~ the respective individual, tissue, or cell is imaged.

55. The ~~use of claim 54~~method of claim 50, wherein the ~~medicament is~~vector of a) and/or the cells of ~~b)~~ are provided to the individual before ~~at~~ the therapy, during ~~at~~ the therapy, and/or after ~~at~~ the therapy.

56. A nucleic acid molecule encoding the poly A aptamer polynucleotide comprising in a 5' to 3' direction:

- a) a 5' splice donor site;
- b) an engineered intron;
- c) a first 3' splice acceptor site;
- d) a polyA switch comprising two or more ligand-binding aptamers with one or more ligand binding pockets, and at least one polyA cleavage signal therein;
- e) a second 3' splice acceptor site; and
- f) a nucleic acid sequence encoding an expressible polypeptide.

57. The nucleic acid molecule of claim 56, wherein the nucleic acid is DNA.

58. The nucleic acid molecule of claim 56, wherein the nucleic acid is RNA.

59. A vector for delivery of the nucleic acid of claim 56.

60. The vector of claim 59, wherein the vector is a viral vector.

61. The vector of claim 59, wherein the vector is selected from an adenoviral vector, a lentiviral vector; an adeno-associated viral vector, a *poliovirus* vector, and a retrovirus vector.