

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 examples, the present disclosure provides compositions and methods for the regulation of gene expression using nucleic acid constructs. In some examples, the present disclosure recognizes the utility of alternative splicing in regulation of gene expression in a nucleic acid construct. In some examples, 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 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, wherein in the absence of a ligand bound to the ligand-binding aptamer of the polyA

switch, splicing occurs between the 5' splice donor site and the first 3' splice acceptor site, resulting in degradation of the nucleic acid sequence encoding the expressible polypeptide, and wherein in the presence of a ligand bound to the ligand-binding aptamer of the polyA switch, splicing occurs between the 5' splice donor site and the second 3' splice acceptor site, resulting in an mRNA comprising the nucleic acid sequence encoding the expressible polypeptide.

[0004b] In another aspect, the present disclosure refers to a vector for delivery of a system for modulating gene expression, wherein the vector comprises 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 in an individual, the system comprising in a 5' to 3' direction: a) a 5' splice donor site; b) an 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, wherein in the absence of a ligand bound to the ligand-binding aptamer of the polyA switch, splicing occurs between the 5' splice donor site and the first 3' splice acceptor site, resulting in degradation of the gene product in the cell, and wherein in the presence of a ligand bound to the ligand-binding aptamer of the polyA switch, splicing occurs between the 5' splice donor site and the second 3' splice acceptor site, resulting in expression of the gene product in the cell.

[0004d] In another aspect, the present disclosure refers to an *in vitro* method of modulating expression of a gene product in a cell, the method comprising the step of: introducing into the cell a system comprising in a 5' to 3' direction: a) a 5' splice donor site; b) an 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, wherein in the absence of a ligand bound to the ligand-binding aptamer of the polyA switch, splicing occurs between the 5' splice donor site and the first 3' splice acceptor site, resulting in degradation of the gene product in the cell, and wherein in the presence of a ligand bound to the ligand-

binding aptamer of the polyA switch, splicing occurs between the 5' splice donor site and the second 3' splice acceptor site, resulting in expression of the gene product in the cell.

[0004e] 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 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, wherein in the absence of a ligand bound to the ligand-binding aptamer of the polyA switch, splicing occurs between the 5' splice donor site and the first 3' splice acceptor site, resulting in degradation of the gene product in the cell, and wherein in the presence of a ligand bound to the ligand-binding aptamer of the polyA switch, splicing occurs between the 5' splice donor site and the second 3' splice acceptor site, resulting in expression of the gene product in the cell.

[0004f] In another aspect, the present disclosure refers to a vector for delivery of a nucleic acid molecule for modulating gene expression, wherein the vector comprises the nucleic acid as disclosed herein.

[0005] In some examples, 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 examples, a polyA aptamer polynucleotide of the present disclosure comprises two ligand-binding aptamers. In some examples, a polyA aptamer

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 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,wherein in the absence of a ligand bound to the ligand-binding aptamer of the polyA switch, splicing occurs between the 5' splice donor site and the first 3' splice acceptor site, resulting in degradation of the nucleic acid sequence encoding the expressible polypeptide, and
wherein in the presence of a ligand bound to the ligand-binding aptamer of the polyA switch, splicing occurs between the 5' splice donor site and the second 3' splice acceptor site, resulting in an mRNA comprising the nucleic acid sequence encoding the 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, 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, 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 9, wherein one or more nucleotides of the polyA cleavage signal are within the 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 5, 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 5'UTR has a sequence set forth in 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 second 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 at least 2 MAZ sequences.

35. The system of claim 33, wherein the G rich-region comprises 4 MAZ sequences.
36. The system of claim 1, wherein the intron has a sequence of between 100 and 200 bases in length.
37. The system of claim 1, wherein the intron has sequence SEQ ID NO.: 1.
38. The system of claim 1, where the intron is followed by a nucleic acid triplet sequence that modulates the strength of the intron splicing.
39. The system of claim 37, wherein the nucleic acid triplet sequence is a sequence selected from: TTT, TGA, TCT, TAC, CAC, and CAT.
40. The system of claim 1, wherein the system comprises a sequence selected from the group SEQ ID NO.:6 to SEQ ID NO.: 56.
41. 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.
42. A vector for delivery of a system for modulating gene expression, wherein the vector comprises the system of claim 1.
43. The vector of claim 41, wherein the vector is a viral vector.
44. 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.

45. Use of a composition comprising a system for manufacture of a medicament for modulating expression of a gene product in a cell in an individual, the system comprising in a 5' to 3' direction:

- a) a 5' splice donor site
- b) an 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,

wherein in the absence of a ligand bound to the ligand-binding aptamer of the polyA switch, splicing occurs between the 5' splice donor site and the first 3' splice acceptor site, resulting in degradation of the gene product in the cell, and

wherein in the presence of a ligand bound to the ligand-binding aptamer of the polyA switch, splicing occurs between the 5' splice donor site and the second 3' splice acceptor site, resulting in expression of the gene product in the cell.

46. An *in vitro* method of modulating expression of a gene product in a cell, the method comprising the step of:

introducing into the cell a system comprising in a 5' to 3' direction:

- a) a 5' splice donor site
- b) an 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,

wherein in the absence of a ligand bound to the ligand-binding aptamer of the polyA switch, splicing occurs between the 5' splice donor site and the first 3' splice acceptor site, resulting in degradation of the gene product in the cell, and

wherein in the presence of a ligand bound to the ligand-binding aptamer of the polyA switch, splicing occurs between the 5' splice donor site and the second 3' splice acceptor site, resulting in expression of the gene product in the cell.

47. The use of claim 45 or the method of claim 46, wherein the gene product is exogenous to the cell.

48. The use or method of claim 47, wherein the system further comprises a nucleic acid sequence encoding the gene product immediately 3' of the splice site of e).
49. The use of claim 45 or the method of claim 46, wherein the gene product is endogenous to the cell.
50. The use or method of claim 49, wherein the ligand is not provided to the cell to inhibit expression of the endogenous gene product.
51. The use of claim 45 or the method of claim 46, wherein the system further comprises a promoter 5' of the splice site of a).
52. The use or method of claim 51, wherein the promoter is a CMV promoter.
53. The use of any one of claims 45 and 47-52, wherein the ligand is glucose, the individual has diabetes, pre-diabetes, or complications from diabetes, and/or the expressible polynucleotide is insulin.
54. The method of claim 46, wherein the ligand is glucose and/or the expressible polynucleotide is insulin.
55. The use of any one of claims 45 and 47-52 or the method of any one of claims 46-52, wherein the ligand is the gene product of a cancer biomarker, and the expressible polynucleotide is a suicide gene.
56. The use of any one of claims 45 and 47-55 or the method of any one of claims 46-52 and 54-55, wherein 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.

57. The use of any one of claims 45 and 47-52 or the method of any one of claims 46-52, wherein the expressible polynucleotide encodes a detectable gene product, and the respective individual, tissue, or cell is imaged.

58. The use of claim 57, wherein the medicament is provided to the individual before a therapy, during a therapy, and/or after a therapy.

59. 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 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,

wherein in the absence of a ligand bound to the ligand-binding aptamer of the polyA switch, splicing occurs between the 5' splice donor site and the first 3' splice acceptor site, resulting in degradation of the nucleic acid sequence encoding the expressible polypeptide, and

wherein in the presence of a ligand bound to the ligand-binding aptamer of the polyA switch, splicing occurs between the 5' splice donor site and the second 3' splice acceptor site, resulting in an mRNA comprising the nucleic acid sequence encoding the expressible polypeptide.

60. The nucleic acid molecule of claim 59, wherein the nucleic acid is DNA.

61. The nucleic acid molecule of claim 59, wherein the nucleic acid is RNA.

62. A vector for delivery of a nucleic acid molecule for modulating gene expression, wherein the vector comprises the nucleic acid of claim 59.

63. The vector of claim 62, wherein the vector is a viral vector.
64. The vector of claim 62, wherein the vector is selected from an adenoviral vector, a lentiviral vector; an adeno-associated viral vector, a *poliovirus* vector, and a retrovirus vector.