by regulating the level of expression and its timing. A regulated system to control protein expression has practical and, in some cases, essential roles for safe and effective therapeutic applications.

SUMMARY

[0001] In one aspect, there is provided a polynucleotide cassette for the regulation of the expression of a target gene comprising (a) a riboswitch and (b) an alternatively-spliced exon, flanked by a 5′ intron and a 3′ intron, wherein the riboswitch comprises (i) an effector region comprising a stem having 7 to 20 base pairs that includes the 5′ splice site sequence of the 3′ intron, and (ii) an aptamer, wherein: the 5' splice site sequence of the 3' intron comprises the sequence GTAATG or GTRAGT, wherein R is A or G; and the alternatively-spliced exon comprises a stop codon that is in-frame with the target gene when the alternatively-spliced exon is spliced into the target gene mRNA. In one example, the aptamer specifically binds a small molecule ligand.

[0002] In one example, the polynucleotide for the regulation of the expression of a target gene ("gene regulation cassette" "regulatory cassette" or "polynucleotide cassette") contains 5′ and 3′ introns that are derived from an endogenous intron from the target gene. In one example, the 5′ and 3′ introns are exogenous to the target gene. In one example, the 5′ and 3′ introns are derived from intron 2 of the human β-globin gene. In one example, the 5′ intron comprises a stop codon in-frame with the target gene. In one example, the 5′ and 3′ introns are each independently from about 50 to about 300 nucleotides in length. In one example, the 5′ and 3′ introns are each independently from about 125 to about 240 nucleotides in length. In one example, the 5′ and/or 3′ introns have been modified to include, or alter the sequence of, an intron splice enhancer, an intron splice enhancer, a 5′ splice site, a 3′ splice site, or the branch point sequence.

[0003] In one example, the effector region stem of the riboswitch is about 7 to about 20 base pairs in length. In one example, the effector region stem is 8 to 11 base pairs in length.

[0004] In one example, the alternatively-spliced exon is derived from exon 2 of the human dihydrofolate reductase gene (DHFR), mutant human Wilms tumor 1 exon 5, mouse calcium/calmodulin-dependent protein kinase II delta exon 16, or SIRT1 exon 6. In one example, the alternatively-spliced exon is the modified DHFR exon 2 from SEQ ID NO:15. In one example, the alternatively-spliced exon has been modified in one or more of the group consisting of altering the sequence of an exon splice silencer, altering the

sequence of an exon splice enhancer, adding an exon splice enhancer, and adding an exon splice donor. In one example, the alternatively-spliced exon is synthetic (i.e., not derived from a naturally-occurring exon).

[0005] In another aspect, there is provided the polynucleotide cassette of the present invention for use of regulation of the expression of the target gene in therapy. In one example, there is provided use of the polynucleotide cassette as described herein for modulating the expression of a target gene, wherein (a) the polynucleotide cassette of the present invention (as, e.g., described above and herein) is to be inserted into the target gene, (b) the target gene comprising the polynucleotide cassette is to be introduced into a cell, and (c) the cell is to be exposed to a small molecule ligand that specifically binds the aptamer in an amount effective to induce expression of the target gene.

[0006] In one example, expression of the target gene is greater than about 5-fold higher when the small molecule ligand is present than the expression levels when the small molecule ligand is absent. In one example, the expression of the target gene is greater than about 10-fold higher when the small molecule ligand is present than the expression levels when the small molecule ligand is absent.

[0007] In one example, the polynucleotide cassette is inserted into the protein coding region of the target gene. In one example, two or more of the polynucleotide cassettes are inserted into the target gene. In one example, the two or more polynucleotide cassettes comprise different aptamers that specifically bind to different small molecule ligands. In another example, the two or more polynucleotide cassettes comprise the same aptamer of different aptamers that specifically bind the same ligand.

[0008] In one example, the target gene comprising the polynucleotide cassette is incorporated in a vector for the expression of the target gene. In one example, the vector is a viral vector. In further examples, the viral vector is selected from the group consisting of adenoviral vector, adeno-associated virus vector, and lentiviral vector.

[0009] In another aspect, there is provided a vector comprising a target gene that contains a polynucleotide cassette, for use in the regulation of the expression of the target gene in therapy, wherein (a) the vector is to be introduced into the eye; and (b)

a small molecule ligand in an amount effective to induce expression of the target gene is to be provided to the mammal; wherein the polynucleotide cassette comprises (i) a riboswitch and (ii) an alternatively-spliced exon flanked by a 5′ intron and a 3′ intron, wherein the riboswitch comprises an effector region comprising a stem having 7 to 20 base pairs that includes the 5′ splice site sequence of the 3′ intron, and an aptamer that binds a small molecule ligand, wherein: i. the 5' splice site sequence of the 3' intron comprises the sequence GTAATG or GTRAGT, wherein R is A or G; and ii. the alternatively-spliced exon comprises a stop codon that is in-frame with the target gene. In one example, the ligand is a small molecule.

[0010] In one example, the vector is introduced into the eye by intraocular injection. In one example, the vector is a viral vector. In one example, the viral vector is selected from the group consisting of adenoviral vector, adeno-associated virus vector, and lentiviral vector.

[0011] In one example, the polynucleotide for the regulation of the expression of a target gene in the eye contains 5′ and 3′ introns that are derived from an endogenous intron from the target gene. In one example, the 5′ and 3′ introns are exogenous to the target gene. In one example, the 5′ and 3′ introns are derived from intron 2 of the human β-globin gene. In one example, the 5′ intron comprises a stop codon in-frame with the target gene. In one example, the 5′ and 3′ introns are each independently from about 50 to about 300 nucleotides in length. In one example, the 5′ and 3′ introns are each independently from about 125 to about 240 nucleotides in length. In one example, the 5′ and/or 3′ introns have been modified to include, or alter the sequence of, an intron splice enhancer, an intron splice enhancer, a 5′ splice site, a 3′ splice site, or the branch point sequence. In one example, the effector region stem of the riboswitch is about 7 to about 20 base pairs in length. In one example, the effector region stem is 8 to 11 base pairs in length. In one example, the alternatively-spliced exon is derived from exon 2 of the human dihydrofolate reductase gene (DHFR) mutant human Wilms tumor 1 exon 5, mouse calcium/calmodulin-dependent protein kinase II delta exon 16, or SIRT1 exon 6. In one example, the alternatively-spliced exon is the modified DHFR exon 2 from SEQ ID NO:15, a modified exon 2 from human DHFR. In one example, the alternatively-spliced exon has been modified in one or more of the group consisting of altering the sequence of an exon splice silencer, altering the sequence of an exon splice enhancer, adding an exon splice enhancer, and adding an exon splice donor. In one

example, the alternatively-spliced exon is synthetic (i.e., not derived from a naturally-occurring exon).

[0012] In one aspect, there is provided a recombinant polynucleotide comprising a target gene containing the polynucleotide cassette of the present invention. In one example, the polynucleotide cassette is located in the protein coding sequence of the target gene. In one aspect, there is provided an *in vitro* method of modulating the expression of a target gene, the method comprising: a. inserting the polynucleotide cassette of the present invention into a target gene, b. introducing the target gene comprising the polynucleotide cassette into a cell, and c. exposing the cell to a small molecule ligand that specifically binds the aptamer in an amount effective to induce expression of the target gene. In another aspect, there is provided use of the polynucleotide cassette of the present invention in the manufacture of a medicament for the treatment of a disease selected from the group consisting of: cystic fibrosis, hemophilia, muscular dystrophy, thalassemia, and sickle cell anemia.

Claims:

1. A polynucleotide cassette for the regulation of the expression of a target gene comprising

a. a riboswitch

b. an alternatively-spliced exon, flanked by a 5′ intron and a 3′ intron,

wherein the riboswitch comprises (i) an effector region comprising a stem having 7 to 20 base pairs that includes the 5′ splice site sequence of the 3′ intron, and (ii) an aptamer, wherein:

the 5' splice site sequence of the 3' intron comprises the sequence GTAATG or GTRAGT, wherein R is A or G; and

the alternatively-spliced exon comprises a stop codon that is in-frame with the target gene when the alternatively-spliced exon is spliced into the target gene mRNA.

2. The polynucleotide cassette of claim 1, wherein the aptamer binds a small molecule ligand.

3. The polynucleotide cassette of claim 1 or 2 for use in the regulation of the expression of the target gene in therapy.

4. The polynucleotide cassette for use of claim 3, wherein

a. the polynucleotide cassette of claim 1 or 2 is to be inserted into a target gene,

b. the target gene comprising the polynucleotide cassette of claim 1 or 2 is to be introduced into a cell, and

c. the cell is to be exposed to a small molecule ligand that specifically binds the aptamer in an amount effective to induce expression of the target gene.

5. The polynucleotide cassette for use of claim 4, wherein the polynucleotide cassette is to be inserted into the protein coding region of the target gene.

6. The polynucleotide cassette for use of claim 4 or 5, wherein the expression of the target gene is greater than about 5-fold higher, or is greater than about 10-fold higher when the small molecule ligand is present than the expression levels when the small molecule ligand is absent.

7. The polynucleotide cassette for use of any one of claims 4-6, wherein two or more of the polynucleotide cassettes are to be inserted into the target gene.

8. The polynucleotide cassette for use of claim 7, wherein the two or more polynucleotide cassettes comprise different aptamers that specifically bind to different small molecule ligands.

9. The polynucleotide cassette for use of claim 7, wherein the two or more polynucleotide cassettes comprise the same aptamer.

10. The polynucleotide cassette for use of any one of claims 4 to 9, wherein the target gene comprising the polynucleotide cassette is to be incorporated in a vector for the expression of the target gene.

11. A vector comprising a target gene that contains a polynucleotide cassette, for use in the regulation of the expression of the target gene in therapy, wherein:

a. the vector is to be introduced into the eye; and

b. a small molecule ligand in an amount effective to induce expression of the target gene is to be provided to the mammal;

wherein the polynucleotide cassette comprises (i) a riboswitch and (ii) an alternatively-spliced exon flanked by a 5′ intron and a 3′ intron, wherein the riboswitch comprises an effector region comprising a stem having 7 to 20 base pairs that includes the 5′ splice site sequence of the 3′ intron, and an aptamer that binds a small molecule ligand, wherein:

i. the 5' splice site sequence of the 3' intron comprises the sequence GTAATG or GTRAGT, wherein R is A or G; and

ii. the alternatively-spliced exon comprises a stop codon that is in-frame with the target gene.

12. The vector for use of claim 11, wherein the vector is to be introduced into the eye by intraocular injection.

13. The vector for use of claim 11 or 12, wherein the vector is a viral vector.

14. The vector for use of claim 13, wherein the viral vector is selected from the group consisting of adenoviral vector, adeno-associated virus vector, and lentiviral vector.

15. The polynucleotide cassette for use of any one of claims 3 to 10, or the vector for use of any one of claims 11 to 14, wherein the polynucleotide cassette is located in the protein coding sequence of the target gene.

16. The polynucleotide cassette of claim 1 or 2, or the polynucleotide cassette for use of any one of claims 3 to 10 and 15, or the vector for use of any one of claims 11 to 15, wherein the 5′ and 3′ introns

(i) are derived from an endogenous intron from the target gene;

(ii) are exogenous to the target gene; or

(iii) are derived from intron 2 of the human β-globin gene.

17. The polynucleotide cassette of claim 1, 2 or 16, or the polynucleotide cassette for use of any one of claims 3 to 10, 15 and 16, or the vector for use of any one of claims 11 to 16, wherein the 5′ intron comprises a stop codon in-frame with the target gene.

18. The polynucleotide cassette of claim 1, 2, 16 or 17, or the polynucleotide cassette for use of any one of claims 3 to 10 and 15 to 17, or the vector for use of any one of claims 11 to 17, wherein the 5′ and 3′ introns are each independently from about 50 to about 300 nucleotides in length.

19. The polynucleotide cassette, or the polynucleotide cassette for use, or the vector for use of claim 18, wherein the 5′ and 3′ introns are each independently from about 125 to about 240 nucleotides in length.

20. The polynucleotide cassette of any one of claims 1, 2, and 16-19, or the polynucleotide cassette for use of any one of claims 3 to 10 and 15 to 19, or the vector for use of any one of claims 11 to 19, wherein the effector region stem is 8 to 11 base pairs in length.

21. The polynucleotide cassette of any one of claims 1, 2, and 16-20, or the polynucleotide cassette for use of any one of claims 3 to 10 and 15 to 20, or the vector for use of any one of claims 11 to 20, wherein the alternatively-spliced exon

(i) is derived from exon 2 of the human dihydrofolate reductase gene, mutant human Wilms tumor 1 exon 5, mouse calcium/calmodulin-dependent protein kinase II delta exon 16, or SIRT1 exon 6;

(ii) has been modified by one or more of the group consisting of altering the sequence of an exon splice enhancer, altering the sequence of exon splice silencer, adding an exon splice enhancer, and adding an exon splice silencer;

(iii) is the modified DHFR exon 2 from SEQ ID NO:15; or

(iv) is synthetic.

22. A recombinant polynucleotide comprising a target gene containing the polynucleotide cassette of any one of claims 1 to 2 and 16 to 21.

23. The recombinant polynucleotide of claim 22, wherein the polynucleotide cassette is located in the protein coding sequence of the target gene.

24. A vector comprising a target gene that contains a polynucleotide cassette of any one of claims 1 to 2 and 16 to 21.

25. The vector of claim 24, wherein the vector is a viral vector.

26. The vector of claim 25, wherein the viral vector is selected from the group consisting of adenoviral vector, adeno-associated virus vector, and lentiviral vector.

27. An *in vitro* method of modulating the expression of a target gene, the method comprising:

a. inserting the polynucleotide cassette of claim 1 or 2 into a target gene,

b. introducing the target gene comprising the polynucleotide cassette into a cell, and

c. exposing the cell to a small molecule ligand that specifically binds the aptamer in an amount effective to induce expression of the target gene.

28. Use of the polynucleotide cassette of any one of claims 1, 2, and 16-21 in the manufacture of a medicament for the treatment of a disease selected from the group consisting of: cystic fibrosis, hemophilia, muscular dystrophy, thalassemia, and sickle cell anemia.