interferon alpha fusion protein and demonstrate that the fusion protein had an approximate 140-fold slower clearance such that the half-life of the fusion was 18-fold longer than for the interferon alpha protein alone. Other examples of therapeutic proteins recently under development that are albumin fusions include Albulin-G™, Cardeva™ and Albugranin™ (Teva Pharmaceutical Industries, fused to Insulin, b-type natriuretic, or GCSF, respectively), Syncria® (GlaxoSmithKline, fused to Glucagon-like peptide-1) and Albuferon α-2B, fused to IFN-alpha (see *Current Opinion in Drug Discovery and Development*, (2009), vol 12, No. 2. p. 288). In these cases, Albulin-G™, Cardeva™ and Syncria® are all fusion proteins where the albumin is found on the N-terminus of the fusion, while Albugranin™ and Albuferon alpha 2G are fusions where the albumin is on the C-terminus of the fusion.

[0015] Thus, there remains a need for additional methods and compositions that can be used to express a desired transgene at a therapeutically relevant level, while avoiding any associated toxicity, and which may limit expression of the transgene to the desired tissue type, for example to treat genetic diseases such as hemophilias, diabetes, lysosomal storage diseases and A1AT deficiency. Additionally, there remains a need for additional methods and compositions to express a desired transgene at a therapeutically relevant level for the treatment of other diseases such as cancers.

SUMMARY

[0016] Disclosed herein are methods and compositions for creating a safe harbor in the genome of cells, for targeted insertion and subsequence expression of a transgene, for example expression of the transgene from a secretory tissue such as liver. In one aspect, the present invention refers to a non-naturally occurring fusion protein comprising a zinc finger protein or a TAL-effector domain protein that binds to an endogenous albumin gene and a cleavage domain, wherein the fusion protein modifies the endogenous albumin gene, and wherein the zinc finger protein comprises 4, 5 or 6 zinc finger domains comprising a recognition helix region, wherein the zinc finger proteins comprise the recognition helix regions shown in a single row of Table 1, Table 3, Table 5 or Table 8 or the TAL-effector domain protein that binds to a target site as shown in Table 12. Also described herein is a non-naturally occurring zinc-finger protein (ZFP) that binds to target site in a region of interest (*e.g.*, an albumin gene) in a genome, wherein the ZFP comprises one or more engineered zinc-

finger binding domains. In one example, the ZFP is a zinc-finger nuclease (ZFN) that cleaves a target genomic region of interest, wherein the ZFN comprises one or more engineered zinc-finger binding domains and a nuclease cleavage domain or cleavage half-domain. Cleavage domains and cleavage half domains can be obtained, for example, from various restriction endonucleases and/or homing endonucleases. In one example, the cleavage half-domains are derived from a Type IIS restriction

described herein, operably linked to a promoter. In one example, the expression vector is a viral vector. In one example, the viral vector exhibits tissue specific tropism.

[0021] In another aspect, the present invention refers to an isolated cell comprising one or more fusion proteins according to the present invention or one or more polynucleotides according to the present invention. Also, described herein is a host cell comprising one or more ZFN and/or TALEN expression vectors. The host cell may be stably transformed or transiently transfected or a combination thereof with one or more ZFP or TALEN expression vectors. In one example, the host cell is an embryonic stem cell. In other examples, the one or more ZFP and/or TALEN expression vectors express one or more ZFNs and/or TALENs in the host cell. In another example, the host cell may further comprise an exogenous polynucleotide donor sequence. Non-limiting examples of suitable host cells include eukaryotic cells or cell lines such as secretory cells (*e.g.,* liver cells, mucosal cells, salivary gland cells, pituitary cells, etc.), blood cells (red blood cells), stem cells, etc. In any of the examples described herein the host cell can comprise an embryo cell, for example, of a mouse, rat, rabbit or other mammal cell embryo.

[0022] In another aspect, the present invention refers to *in vitro* method of cleaving an endogenous albumin gene in a cell, the method comprising introducing, into the cell, one or more expression vectors comprising at least one fusion protein according to the present invention or at least one polynucleotide according to the present invention, under conditions such that the one or more fusion proteins are expressed and the albumin gene is cleaved. Also, described herein is a method for cleaving an albumin gene in a cell, the method comprising: introducing, into the cell, one or more polynucleotides encoding one or more ZFNs and/or TALENs that bind to a target site in the one or more albumin genes under conditions such that the ZFN(s) is (are) or TALENs is (are) expressed and the one or more albumin genes are cleaved.

[0023] In other examples, a genomic sequence in any target gene is replaced, for example using a ZFN or TALEN (or vector encoding said ZFN or TALEN) as described herein and a "donor" sequence (*e.g.,* transgene) that is inserted into the gene following targeted cleavage with the ZFN and/or TALEN. The donor sequence may be present in the ZFN or TALEN vector, present in a separate vector (*e.g.*, Ad or LV vector) or, alternatively, may be introduced into the cell using a

endogenous target locus or, alternatively from albumin-encoding sequences on the transgene). In some instances, the albumin sequences will be present on the amino (N)-terminal portion of the exogenous protein, while in others, the albumin sequences will be present on the carboxy (C)- terminal portion of the exogenous protein. In other instances, albumin sequences will be present on both the N- and C-terminal portions of the exogenous protein. The albumin sequences may include full-length wild-type or mutant albumin sequences or, alternatively, may include partial albumin amino acid sequences. In certain examples, the albumin sequences (full-length or partial) serve to increase the serum half-life of the polypeptide expressed by the transgene to which it is fused and/or as a carrier. In some examples, the albumin-transgene fusion is located at the endogenous locus within the cell while in other embodiments, the albumin-transgene coding sequence is inserted into a safe harbor within a genome. In some examples, the safe harbor is selected from the AAVS1, Rosa, HPRT or CCR5 locus (see co-owned US patent publications Nos. 20080299580; 20080159996 and 201000218264, and US Provisional patent application No. 61/556,691).

[0023a] In one aspect, the present invention refers to a non-naturally occurring fusion protein of the present invention or a polynucleotide of the present invention for use in therapy. In another aspect, the present invention refers to a non-naturally occurring fusion protein of the present invention or a polynucleotide of the present invention, and a donor polynucleotide comprising a transgene encoding a therapeutic protein for use in therapy. In yet another aspect, the present invention refers to use of one or more expression vectors comprising at least one fusion protein according to the present invention or at least one polynucleotide according to the present invention in the manufacture of a medicament for treating a lysosomal storage disease, epidermolysis bullosa, AAT deficient emphysema or a blood disorder, wherein the vector or the polynucleotide is introduced into the cell under conditions such that the one or more fusion proteins are expressed and an endogenous albumin gene is cleaved.

[0025] In another example, the invention disclosure methods and compositions that can be used to express a transgene under the control of an albumin promoter *in vivo* (*e.g.,* endogenous or exogenous albumin promoter). In some examples, the transgene may encode a therapeutic protein of interest. The transgene may encode a protein such that the methods disclosed herein can be used for

production of protein that is deficient or lacking (*e.g.*, "protein replacement"). In some instances, the protein may be involved treatment for a lysosomal storage disease. Other therapeutic proteins may be expressed, including protein therapeutics for conditions as diverse as epidermolysis bullosa or AAT deficient emphysema. In other examples, the transgene may comprise sequences (*e.g.*, engineered sequences) such that the expressed protein has characteristics which give it novel and desirable features (increased half-life, changed plasma clearance characteristics etc.). Engineered sequences can also include amino acids derived from the albumin sequence. In some examples, the transgenes encode therapeutic proteins, therapeutic hormones, plasma proteins, antibodies and the like. In some examples, the transgenes may encode proteins involved in blood disorders such as clotting disorders. In some examples, the transgenes encode structural nucleic acids (shRNAs, miRNAs and the like).

CLAIMS

What is claimed is:

1. A non-naturally occurring fusion protein comprising a zinc finger protein or a TAL-effector domain protein that binds to an endogenous albumin gene and a cleavage domain, wherein the fusion protein modifies the endogenous albumin gene, and wherein the zinc finger protein comprises 4, 5 or 6 zinc finger domains comprising a recognition helix region, wherein the zinc finger proteins comprise the recognition helix regions shown in a single row of Table 1, Table 3, Table 5 or Table 8 or the TAL-effector domain protein that binds to a target site as shown in Table 12.

2. A polynucleotide encoding one or more fusion proteins of claim 1.

3. An isolated cell comprising one or more fusion proteins according to claim 1 or one or more polynucleotides according to claim 2.

4. The cell of claim 3, wherein the cell is a stem cell.

5. The cell of claim 4, wherein the stem cell is selected from the group consisting of an embryonic stem cell (ESC), an induced pluripotent stem cell (iPSC), a hepatic stem cell and a liver stem cell.

6. A kit comprising a fusion protein according to claim 1 or a polynucleotide according to claim 2.

7. An *in vitro* method of cleaving an endogenous albumin gene in a cell, the method comprising:

introducing, into the cell, one or more expression vectors comprising at least one fusion protein according to claim 1 or at least one polynucleotide according to claim 2, under conditions such that the one or more fusion proteins are expressed and the albumin gene is cleaved.

8. The method of claim 7, wherein the polynucleotide comprises an AAV vector.

9. The method of claim 7 or claim 8, wherein the cell is a liver cell.

10. A non-naturally occurring fusion protein of claim 1 or a polynucleotide of claim 2 for use in therapy.

11. A non-naturally occurring fusion protein of claim 1 or a polynucleotide of claim 2, and a donor polynucleotide comprising a transgene encoding a therapeutic protein for use in therapy.

12. Use of one or more expression vectors comprising at least one fusion protein according to claim 1 or at least one polynucelotide according to claim 2 in the manufacture of a medicament for treating a lysosomal storage disease, epidermolysis bullosa, AAT deficient emphysema or a blood disorder, wherein the vector or the polynucleotide is introduced into the cell under conditions such that the one or more fusion proteins are expressed and an endogenous albumin gene is cleaved.

13. The use of claim 12, wherein the blood disorder is a clotting disorder.

14. The use of claim 12 or 13, wherein the medicament further comprises a transgene encoding a therapeutic protein, wherein the transgene is integrated into the albumin gene following cleavage of the albumin gene by a nuclease.

15. The use of claim 14, wherein expression of the transgene is driven by control sequences of the endogenous albumin.

CLAIMS

What is claimed is:

1. A non-naturally occurring fusion protein comprising a zinc finger protein that binds to an endogenous albumin gene and a cleavage domain, wherein the fusion protein modifies the endogenous albumin gene.

2. The fusion protein of claim 2, wherein the zinc finger protein comprises 4, 5 or 6 zinc finger domains comprising a recognition helix region, wherein the zinc finger proteins comprise the recognition helix regions shown in a single row of Table 1, Table 3, Table 5 or Table 8.

3. A polynucleotide encoding one or more fusion proteins of claim 1.

4. An isolated cell comprising one or more fusion proteins according to claim 1 or claim 2 or one or more polynucleotides according to claim 3.

5. The cell of claim 4, wherein the cell is a stem cell.

6. The cell of claim 5, wherein the stem cell is selected from the group consisting of an embryonic stem cell (ESC), an induced pluripotent stem cell (iPSC), a hepatic stem cell and a liver stem cell.

7. A kit comprising a fusion protein according to claim 1 or claim 2 of a polynucleotide according to claim 3.

8. A method of cleaving an endogenous albumin gene in a cell, the method comprising:

introducing, into the cell, one or more expression vectors comprising at least one fusion protein according to claim 1 or claim 2 or at least one polynucleotide according to claim 3, under conditions such that the one or more fusion proteins are expressed and the albumin gene is cleaved.

9. The method of claim 8, wherein the polynucleotide comprises an AAV vector.

10. The method of claim 8 or claim 9, wherein the cell is a liver cell.