METHODS AND COMPOSITIONS RELATED TO LARGE SCALE PRODUCTION OF PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of U.S. Provisional Application No. 61/819,912, filed May 6, 2013, and to U.S. Provisional Application No. 61/954,692, filed March 18, 2014, both of which are hereby incorporated by reference in their entireties.

BACKGROUND

Therapeutic proteins are widely employed in treating various acquired and genetic diseases such as cancer and enzyme deficiencies (Wurm et al. Nat. Biotechnol. 22, 1393 - 1398). There are three major problems impeding even more widespread use. First, they are difficult and expensive to produce. Second, they are often modified by glycosylation or sulfation processes which are poorly reproduced in most mammalian cell culture systems. Third, they often have a short half-life *in vivo*, necessitating frequent injection or infusion. Accordingly, there is a need for more efficient and effective compositions and methods for protein production and use.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides ~~Disclosed herein are~~ nucleic acids encoding a non-albumin protein (also referred to herein as a protein of interest) operably inserted into an albumin gene locus in a hepatocyte or hepatocyte-derived cell line. In another aspect, the present invention provides ~~Also disclosed are~~ nucleic acids encoding a non-albumin protein (protein of interest) operably inserted into a non-endogenous gene selected from the group consisting of: alpha-1-microglobulin/bikunin precursor; alpha-2-HS-glycoprotein; alphafetoprotein; apolipoprotein A2; apolipoprotein C1; apolipoprotein H; fibrinogen gamma chain; serpin peptidase inhibitor, clade A, member 1; serpin peptidase inhibitor, clade A, member 3; serpin peptidase inhibitor, clade A, member 7; serpin peptidase inhibitor, clade C, member 1; and transferrin; wherein the non-endogenous gene is in a hepatocyte or hepatocyte-derived cell line. In yet another aspect, the present invention provides a hepatocyte cell, or hepatocyte-derived cell, comprising the nucleic acid disclosed herein. Also provided is a hepatocyte or hepatocyte-derived cell line lacking endogenous albumin coding sequence, comprising a nucleic acid encoding a non-albumin protein (protein of interest) operably inserted into a non-endogenous gene, for example, the albumin gene locus.

Also disclosed is a protein produced by a nucleic acid encoding a non-albumin protein operably inserted into nucleic acid encoding a non-albumin protein, for example, an albumin gene locus, in a hepatocyte or hepatocyte-derived cell line.

In yet another aspect, the present invention provides a purified protein produced by the cell disclosed herein.

In one aspect, the present invention provides a composition comprising the protein disclosed herein, and a pharmaceutically acceptable carrier.

Also disclosed herein is a system which is useful for producing a protein from the engineered cells disclosed herein. In one aspect, the present invention provides a system useful for producing a protein, wherein the systems comprise cells disclosed herein and an incubator useful for incubating the cells.

In one aspect, the invention provides an *in vitro* ~~Further disclosed herein is a~~ method of producing a non-albumin protein, the method comprising a) culturing the engineered cells disclosed herein; and b) allowing the cell to produce the non-albumin protein.

In one aspect, the present invention provides ~~Disclosed are~~ polypeptides comprising multiple domains, where at least two domains are selected from different members of the albumin superfamily. This is referred to as an SFP, or synthetic fusion protein. In another aspect, the present invention provides nucleic acids encoding the polypeptides disclosed herein (SFP). The SFP can have one, two, three, four, or more domains. The polypeptide can be fused to a protein of interest (POI), and together this molecule is referred to as the SFP-POI. In one aspect, the present invention provides polypeptides (SFP-POI) comprising polypeptides disclosed herein (SFP) and a protein of interest (POI). In another aspect, the present invention provides nucleic acids encoding the polypeptides disclosed herein (SFP-POI).

In yet another aspect, the present invention provides a nucleic acid comprising the nucleic acids disclosed herein, and other specific nucleic acids such that the nucleic acid can be incorporated into a specific target sequence in the genome. In yet another aspect, the present invention provides vectors comprising the nucleic acids described herein. In yet another aspect, the present invention provides host cells comprising the nucleic acids described herein.

In one aspect, the present invention provides kits comprising the polypeptides disclosed herein.

In one aspect, the present invention provides the polypeptide disclosed herein for use in ~~Also disclosed are methods of~~ modulating distribution of a protein of interest within a subject, wherein the polypeptide disclosed herein is to be administered ~~the method comprising administering~~ to the subject and ~~the polypeptide described above, wherein~~ the polypeptide modulates the distribution of the peptide of interest within the subject.

In one aspect, the present invention provides the polypeptide disclosed herein for use in the treatment of ~~Also disclosed is a method of treating~~ a subject with a disease or for use in the prevention of the ~~, or preventing said~~ disease in the subject, wherein the polypeptide disclosed herein is to be administered ~~the method comprising administering~~ to the subject and ~~the polypeptide described herein, wherein~~ the protein of interest is able to treat or prevent the disease.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

Figure 1 shows a computer generated model of SFP compared to the actual structure of human albumin. The triangle marks the conserved histidine triad that is responsible for binding to the neonatal Fc receptor.

Figure 2 shows albumin synthesis in cultures of the C3A cell line.

Figure 3 shows a diagram of insertion into the albumin locus.

Figures 4A-C shows construction of the Factor IX (FIXneo) and Stabile9 (S9neo) targeting plasmids.

Figure 5 shows BChE and StabileBChE targeting plasmids.

Figure 6 shows G418 resistant clones analyzed for insertion into the albumin locus via PCR.

Figure 7 shows clones analyzed for presence of Factor IX in the supernate via ELISA.

Figure 8 shows clones analyzed for Factor IX enzyme activity.

Figure 9 shows clones analyzed for mRNA via QPCR.

Figure 10 shows codon optimization increases production of Factor IX mRNA.

Figure 11 shows the structure of the Factor IX minigene construct.

WHAT IS CLAIMED IS:

1. A polypeptide comprising multiple domains, where at least two domains are selected from different members of the albumin superfamily.
2. The polypeptide of claim 1, wherein the members of the albumin superfamily from which each domain is selected are albumin, alpha-fetoprotein, vitamin D-binding protein and afamin.
3. The polypeptide of claim 1, wherein the polypeptide has three or more domains selected from different members of the albumin superfamily.
4. The polypeptide of claim 1, wherein at least one domain is from vitamin D-binding protein.
5. The polypeptide of claim 4, wherein at least two domains are from vitamin D-binding protein.
6. The polypeptide of claim 5, wherein the polypeptide can bind vitamin D.
7. The polypeptide of claim 1, 4, or 5, wherein at least one domain is selected from alphafetoprotein.
8. The polypeptide of claim 1, wherein at least one domain is selected from afamin.
9. The polypeptide of claim 1, wherein at least two domains are selected from afamin.
10. The polypeptide of claim 1, 8, or 9, wherein at least one domain is selected from alphafetoprotein.
11. The polypeptide of claim 9, wherein the polypeptide can bind vitamin E.
12. The polypeptide of claim 1, wherein each domain of the polypeptide has 80% or greater homology to a domain selected from a member of the albumin superfamily.
13. The polypeptide of claim 1, wherein the entire polypeptide has less than 80% homology to human albumin or alpha-fetoprotein.
14. The polypeptide of claim 1, wherein each individual domain can comprise peptide sequences from more than one member of the human albumin superfamily.
15. The polypeptide of claim 14, wherein the domain can comprise one or more amino acid substitution when compared to the native domain from the albumin superfamily.
16. The polypeptide of claim 15, wherein the substitutions can be non-naturally occurring amino acid substitutions.
17. The polypeptide of claim 15, wherein amino acids are altered to maximize binding to the

neonatal Fc receptor.

18. The polypeptide of claim 15, wherein amino acids are altered to prevent glycosylation.
19. A polypeptide comprising the polypeptide of claim 1 and a protein of interest.
20. The polypeptide of claim 19, wherein the protein of interest is a therapeutic protein
21. The polypeptide of claim 19, wherein the protein of interest is coagulation factor IX.
22. The polypeptide of claim 19, wherein the protein of interest is butyrylcholinesterase.
23. The polypeptide of claim 19, wherein the protein of interest is coagulation factor VIII, coagulation factor VIIa, alpha-1-antitrypsin, antithrombin III, phenylalanine hydroxylase, erythropoietin, growth hormone, granulocyte colony stimulating factor, interferon beta, or atrial natriuretic peptide.
24. The polypeptide of claim 19, wherein the protein of interest is a vaccine antigen.
25. The polypeptide of claim 19, wherein the protein of interest is a single chain variable fragment.
26. The polypeptide of claim 19, wherein the protein of interest is a bispecific antibody
27. A nucleic acid encoding the polypeptide of claim 1.
28. A nucleic acid encoding the polypeptide of claim 19.
29. A nucleic acid comprising the nucleic acid of claim 27 or 28, and other specific nucleic acids such that the nucleic acid can be incorporated into a specific target sequence in the genome.
30. The nucleic acid of claim 29, wherein the specific target sequence is the human albumin gene.
31. The nucleic acid of claim 29, wherein the target sequence is alpha-1-antitrypsin, transferrin, antithrombin III, alpha-fetoprotein, or insulin like growth factor II.
32. A vector comprising the nucleic acid of claim 27 or 28.
33. A host cell comprising the nucleic acid of claim 27 or 28.
34. The host cell of claim 33, wherein the cell is a liver-derived cell.
35. The host cell of claim 34, wherein the cell is a HepG2/C3A cell.
36. The host cell of claim 33, wherein the cell is American Type Culture Collection #CRL-10741.
37. A kit comprising the polypeptide of claim 1.
38. The polypeptide of claim 1, wherein the polypeptide has 20% or greater half-life when compared to native albumin polypeptide.
39. A method of modulating distribution of a peptide of interest within a subject, the method comprising administering to the subject the polypeptide of claim 19, wherein the

polypeptide modulates the distribution of the peptide of interest within the subject.

40. The method of claim 39, wherein the distribution of the peptide of interest is slower.
41. The method of claim 1, wherein the administration of the composition to the subject results in a blood level half-life of the peptide of interest which is greater than the blood level half-life obtained upon administration of the peptide of interest alone.
42. The method of claim 1, wherein the administration of the composition to the subject results in a blood level half-life of the peptide of interest which is greater than the blood level half-life obtained upon administration of the peptide of interest fused to albumin or a derivative thereof.
43. The method of claim 39, wherein said polypeptide is administered to the subject via injection, inhalation, internasally, or orally.
44. A method of treating a subject with a disease, or preventing the disease in the subject, the method comprising administering to the subject the polypeptide of claim 19, wherein the protein of interest is able to treat or prevent the disease.
45. A nucleic acid encoding a non-albumin protein operably inserted into an albumin gene locus in a hepatocyte or hepatocyte-derived cell line.
46. A nucleic acid encoding a non-albumin protein operably inserted into a non-endogenous gene selected from the group consisting of: alpha-1-microglobulin/bikunin precursor; alpha-2-HS-glycoprotein; alphafetoprotein; apolipoprotein A2; apolipoprotein C1; apolipoprotein H; fibrinogen gamma chain; serpin peptidase inhibitor, clade A, member 1; serpin peptidase inhibitor, clade A, member 3; serpin peptidase inhibitor, clade A, member 7; serpin peptidase inhibitor, clade C, member 1; and transferrin; wherein the non-endogenous gene is in a hepatocyte or hepatocyte-derived cell line.
47. A hepatocyte cell, or hepatocyte-derived cell, comprising the nucleic acid of claim 45 or 46.
48. The cell of claim 47, wherein the cell is an immortal liver cell.
49. The cell of claim 48, wherein the liver cell is selected from the group comprising Hep3B2.1-7, HepG2, C3A (HepG2/C3A), HuH-7, and HuH-6.
50. The cell of claim 49, wherein the liver cell is a C3A cell.
51. The nucleic acid of claim 45 or 46, wherein the non-albumin protein is selected from the list in Table 3.
52. The nucleic acid of claim 51, wherein the non-albumin protein is Factor IX.
53. The nucleic acid of claim 51, wherein the non-albumin protein is Factor VIII.
54. The nucleic acid of claim 51, wherein the non-albumin protein is selected from the group

consisting of erythropoietin, thrombopoietin, stem cell factor (KIT ligand), interleukin 3, interleukin 50, insulin, and flt3.

55. The cell of claim 47, where the cell produces at least 0.1 picograms/cell/day.
56. The cell of claim 47, where the cell produces at least 20 picograms/cell/day.
57. The cell of claim 47, wherein the cell is optimized for production of the non-albumin protein by optimization of coding sequence of cDNA encoding the non-albumin protein.
58. The cell of claim 47, wherein the cell is optimized for production of the non-albumin protein by inclusion of an intervening sequence in the nucleic acid encoding the non-albumin protein.
59. The cell of claim 47, wherein the cell is optimized for production of the non-albumin protein by inclusion of a stabilizing 3' untranslated region within the nucleic acid encoding the non-albumin protein.
60. A purified protein produced by the cell of claim 47.
61. A purified protein produced by the cell of claim 47, wherein the protein has a human glycosylation pattern.
62. The purified protein of claim 60, wherein the protein is Factor IX.
63. The purified protein of claim 60, wherein the protein is Factor VIII.
64. A composition comprising the protein of claim 61, and a pharmaceutically acceptable carrier.
65. The composition of claim 64, wherein the pharmaceutically acceptable carrier allows for intravenous administration, intraperitoneal administration, intramuscular administration, intracoronary administration, intraarterial administration, intradermal administration, subcutaneous administration, transdermal delivery, intratracheal administration, subcutaneous administration, intraarticular administration, intraventricular administration, inhalation, intracerebral, nasal, naval, oral, intraocular, pulmonary administration, impregnation of a catheter, by suppository and direct injection into a tissue, or systemically absorbed topical or mucosal administration.
66. A system useful for producing a protein, wherein the system comprises cells of claim 47 and an incubator useful for incubating the cells.
67. The cell of claim 47, further comprising a second nucleic acid encoding a protein operably inserted into a gene which is non-endogenous to the second nucleic acid.
68. The cell of claim 67, wherein the second nucleic acid is different than the first nucleic acid.
69. The cell of claim 67, wherein the first and second nucleic acids are inserted into different

non-endogenous genes.

70. The cell of claim 67, wherein the non-endogenous gene is albumin.
71. The cell of claim 67, wherein the non-endogenous gene is not albumin.
72. The cell of claim 71, wherein the non-endogenous gene is selected from the group comprising alpha-1-microglobulin/bikunin precursor; alpha-2-HS-glycoprotein; alphafetoprotein; apolipoprotein A2; apolipoprotein C1; apolipoprotein H; fibrinogen gamma chain; serpin peptidase inhibitor, clade A, member 1; serpin peptidase inhibitor, clade A, member 3; serpin peptidase inhibitor, clade A, member 7; serpin peptidase inhibitor, clade C, member 1; and transferrin.
73. The cell of claim 67, wherein the second nucleic acid encodes a protein selected from the list in Table 3.
74. A method of producing a non-albumin protein, the method comprising
    a. culturing the cell of claim 47; and
    b. allowing the cell to produce the non-albumin protein.
75. The method of claim 74, wherein the albumin gene is excised prior to producing the non-albumin gene.
76. The method of claim 75, wherein the albumin gene is partially or completely excised.
77. The method of claim 74, wherein albumin is expressed as a fusion protein with the non-albumin protein.
78. The method of claim 74, wherein the cells are cultured in a mobile clean room.
79. The method of claim 74, further comprising the step of:
    c. purifying the non-albumin protein.
80. The method of claim 74, wherein the cell further comprises a second nucleic acid encoding a protein operably inserted into gene which is non-endogenous to the second nucleic acid.
81. The method of claim 74, wherein before the cell is cultured, coding of cDNA encoding the non-albumin protein is optimized.
82. The method of claim 74, wherein before the cell is cultured, an intervening sequence is included in the nucleic acid encoding the non-albumin protein.
83. The method of claim 74, wherein before the cell is cultured, a stabilizing 3' untranslated region is included within the nucleic acid encoding the non-albumin protein
84. The method of any one of claims 81-83, wherein production of said non-albumin protein is at least 2-fold greater when compared to a control, wherein the control comprises the

same non-albumin protein.

85. The method of claim 38, wherein expression is at least 3-fold greater.

WHAT IS CLAIMED IS:

1. A polypeptide comprising multiple domains, where at least two domains are selected from different members of the albumin superfamily.
2. The polypeptide of claim 1, wherein the members of the albumin superfamily from which each domain is selected are albumin, alpha-fetoprotein, vitamin D-binding protein, and afamin.
3. The polypeptide of claim 1, wherein the polypeptide has three or more domains selected from different members of the albumin superfamily.
4. The polypeptide of claim 1, wherein at least one domain is from vitamin D-binding protein.
5. The polypeptide of claim 4, wherein at least two domains are from vitamin D-binding protein.
6. The polypeptide of claim 5, wherein the polypeptide can bind vitamin D.
7. The polypeptide of claim 1, 4, or 5, wherein at least one domain is selected from alphafetoprotein.
8. The polypeptide of claim 1, wherein at least one domain is selected from afamin.
9. The polypeptide of claim 1, wherein at least two domains are selected from afamin.
10. The polypeptide of claim 1, 8, or 9, wherein at least one domain is selected from alphafetoprotein.
11. The polypeptide of claim 9, wherein the polypeptide can bind vitamin E.
12. The polypeptide of claim 1, wherein each domain of the polypeptide has 80% or greater homology to a domain selected from a member of the albumin superfamily.
13. The polypeptide of claim 1, wherein the entire polypeptide has less than 80% homology to human albumin or alpha-fetoprotein.
14. The polypeptide of claim 1, wherein each individual domain can comprise peptide sequences from more than one member of the human albumin superfamily.
15. The polypeptide of claim 14, wherein the domain can comprise one or more amino acid substitution when compared to the native domain from the albumin superfamily.
16. The polypeptide of claim 15, wherein the substitutions can be non-naturally occurring amino acid substitutions.
17. The polypeptide of claim 15, wherein amino acids are altered to maximize binding to the neonatal Fc receptor.

18. The polypeptide of claim 15, wherein amino acids are altered to prevent glycosylation.
19. A polypeptide comprising the polypeptide of claim 1 and a protein of interest.
20. The polypeptide of claim 19, wherein the protein of interest is a therapeutic protein
21. The polypeptide of claim 19, wherein the protein of interest is coagulation factor IX.
22. The polypeptide of claim 19, wherein the protein of interest is butyrylcholinesterase.
23. The polypeptide of claim 19, wherein the protein of interest is coagulation factor VIII, coagulation factor VIIa, alpha-1-antitrypsin, antithrombin III, phenylalanine hydroxylase, erythropoietin, growth hormone, granulocyte colony stimulating factor, interferon beta, or atrial natriuretic peptide.
24. The polypeptide of claim 19, wherein the protein of interest is a vaccine antigen.
25. The polypeptide of claim 19, wherein the protein of interest is a single chain variable fragment.
26. The polypeptide of claim 19, wherein the protein of interest is a bispecific antibody
27. A nucleic acid encoding the polypeptide of claim 1.
28. A nucleic acid encoding the polypeptide of claim 19.
29. A nucleic acid comprising the nucleic acid of claim 27 or 28, and other specific nucleic acids such that the nucleic acid can be incorporated into a specific target sequence in the genome.
30. The nucleic acid of claim 29, wherein the specific target sequence is the human albumin gene.
31. The nucleic acid of claim 29, wherein the target sequence is alpha-1-antitrypsin, transferrin, antithrombin III, alpha-fetoprotein, or insulin like growth factor II.
32. A vector comprising the nucleic acid of claim 27 or 28.
33. A host cell comprising the nucleic acid of claim 27 or 28.
34. The host cell of claim 33, wherein the cell is a liver-derived cell.
35. The host cell of claim 34, wherein the cell is a HepG2/C3A cell.
36. The host cell of claim 33, wherein the cell is American Type Culture Collection #CRL-10741.
37. A kit comprising the polypeptide of claim 1.
38. The polypeptide of claim 1, wherein the polypeptide has 20% or greater half-life when compared to native albumin polypeptide.
39. The polypeptide of claim 19 for use in modulating distribution of a peptide of interest within a subject, wherein the polypeptide is to be administered to the subject and the polypeptide modulates the distribution of the peptide of interest within the subject.

40. The polypeptide of claim 39, wherein the distribution of the peptide of interest is slower.
41. The polypeptide of claim 39, wherein the administration of the polypeptide to the subject results in a blood level half-life of the peptide of interest which is greater than the blood level half-life obtained upon administration of the peptide of interest alone.
42. The polypeptide of claim 39, wherein the administration of the polypeptide to the subject results in a blood level half-life of the peptide of interest which is greater than the blood level half-life obtained upon administration of the peptide of interest fused to albumin or a derivative thereof.
43. The polypeptide of claim 39, wherein said polypeptide is to be administered to the subject via injection, inhalation, internasally, or orally.
44. The polypeptide of claim 19 for use in the treatment of a subject with a disease or for use in the prevention of the disease in the subject, wherein the polypeptide is to be administered to the subject and the protein of interest is able to treat or prevent the disease.
45. A nucleic acid encoding a non-albumin protein operably inserted into an albumin gene locus in a hepatocyte or hepatocyte-derived cell line.
46. A nucleic acid encoding a non-albumin protein operably inserted into a non-endogenous gene selected from the group consisting of: alpha-1-microglobulin/bikunin precursor; alpha-2-HS-glycoprotein; alphafetoprotein; apolipoprotein A2; apolipoprotein C1; apolipoprotein H; fibrinogen gamma chain; serpin peptidase inhibitor, clade A, member 1; serpin peptidase inhibitor, clade A, member 3; serpin peptidase inhibitor, clade A, member 7; serpin peptidase inhibitor, clade C, member 1; and transferrin; wherein the non-endogenous gene is in a hepatocyte or hepatocyte-derived cell line.
47. A hepatocyte cell, or hepatocyte-derived cell, comprising the nucleic acid of claim 45 or 46.
48. The cell of claim 47, wherein the cell is an immortal liver cell.
49. The cell of claim 48, wherein the liver cell is selected from the group comprising Hep3B2.1-7, HepG2, C3A (HepG2/C3A), HuH-7, and HuH-6.
50. The cell of claim 49, wherein the liver cell is a C3A cell.
51. The nucleic acid of claim 45 or 46, wherein the non-albumin protein is selected from the list in Table 3.
52. The nucleic acid of claim 51, wherein the non-albumin protein is Factor IX.

53. The nucleic acid of claim 51, wherein the non-albumin protein is Factor VIII.
54. The nucleic acid of claim 51, wherein the non-albumin protein is selected from the group consisting of erythropoietin, thrombopoietin, stem cell factor (KIT ligand), interleukin 3, interleukin 50, insulin, and flt3.
55. The cell of claim 47, where the cell produces at least 0.1 picograms/cell/day.
56. The cell of claim 47, where the cell produces at least 20 picograms/cell/day.
57. The cell of claim 47, wherein the cell is optimized for production of the non-albumin protein by optimization of coding sequence of cDNA encoding the non-albumin protein.
58. The cell of claim 47, wherein the cell is optimized for production of the non-albumin protein by inclusion of an intervening sequence in the nucleic acid encoding the non-albumin protein.
59. The cell of claim 47, wherein the cell is optimized for production of the non-albumin protein by inclusion of a stabilizing 3' untranslated region within the nucleic acid encoding the non-albumin protein.
60. A purified protein produced by the cell of claim 47.
61. A purified protein produced by the cell of claim 47, wherein the protein has a human glycosylation pattern.
62. The purified protein of claim 60, wherein the protein is Factor IX.
63. The purified protein of claim 60, wherein the protein is Factor VIII.
64. A composition comprising the protein of claim 61, and a pharmaceutically acceptable carrier.
65. The composition of claim 64, wherein the pharmaceutically acceptable carrier allows for intravenous administration, intraperitoneal administration, intramuscular administration, intracoronary administration, intraarterial administration, intradermal administration, subcutaneous administration, transdermal delivery, intratracheal administration, subcutaneous administration, intraarticular administration, intraventricular administration, inhalation, intracerebral, nasal, naval, oral, intraocular, pulmonary administration, impregnation of a catheter, by suppository and direct injection into a tissue, or systemically absorbed topical or mucosal administration.
66. A system useful for producing a protein, wherein the system comprises cells of claim 47 and an incubator useful for incubating the cells.
67. The cell of claim 47, further comprising a second nucleic acid encoding a protein operably inserted into a gene which is non-endogenous to the second nucleic acid.

68. The cell of claim 67, wherein the second nucleic acid is different than the first nucleic acid.
69. The cell of claim 67, wherein the first and second nucleic acids are inserted into different non-endogenous genes.
70. The cell of claim 67, wherein the non-endogenous gene is albumin.
71. The cell of claim 67, wherein the non-endogenous gene is not albumin.
72. The cell of claim 71, wherein the non-endogenous gene is selected from the group comprising alpha-1-microglobulin/bikunin precursor; alpha-2-HS-glycoprotein; alphafetoprotein; apolipoprotein A2; apolipoprotein C1; apolipoprotein H; fibrinogen gamma chain; serpin peptidase inhibitor, clade A, member 1; serpin peptidase inhibitor, clade A, member 3; serpin peptidase inhibitor, clade A, member 7; serpin peptidase inhibitor, clade C, member 1; and transferrin.
73. The cell of claim 67, wherein the second nucleic acid encodes a protein selected from the list in Table 3.
74. An *in vitro* method of producing a non-albumin protein, the method comprising
    a. culturing the cell of claim 47; and
    b. allowing the cell to produce the non-albumin protein.
75. The method of claim 74, wherein the albumin gene is excised prior to producing the non-albumin gene.
76. The method of claim 75, wherein the albumin gene is partially or completely excised.
77. The method of claim 74, wherein albumin is expressed as a fusion protein with the non-albumin protein.
78. The method of claim 74, wherein the cells are cultured in a mobile clean room.
79. The method of claim 74, further comprising the step of:
    c. purifying the non-albumin protein.
80. The method of claim 74, wherein the cell further comprises a second nucleic acid encoding a protein operably inserted into gene which is non-endogenous to the second nucleic acid.
81. The method of claim 74, wherein before the cell is cultured, coding of cDNA encoding the non-albumin protein is optimized.
82. The method of claim 74, wherein before the cell is cultured, an intervening sequence is included in the nucleic acid encoding the non-albumin protein.
83. The method of claim 74, wherein before the cell is cultured, a stabilizing 3' untranslated region is included within the nucleic acid encoding the non-albumin

protein

84. The method of any one of claims 81-83, wherein production of said non-albumin protein is at least 2-fold greater when compared to a control, wherein the control comprises the same non-albumin protein.

85. The method of claim 38, wherein expression is at least 3-fold greater.