CLAIMS

1. A vertebrate cell for producing a molecule, which naturally comprises fucose on its glycomoieties, lacking fucose or with a reduced amount of fucose on its glycomoieties comprising
at least one enzyme which uses GDP-6-deoxy-D-lyxo-4-hexulose as a substrate, wherein the enzyme does not catalyze the reaction which converts GDP-6-deoxy-D-lyxo-4-hexulose into GDP-L-fucose.

2. The vertebrate cell of claim 1, wherein the enzyme which uses GDP-6-deoxy-D-lyxo-4-hexulose as a substrate is selected from the group consisting of GDP-6-deoxy-D-lyxo-4-hexulose reductase (RMD), GDP-perosamine synthetase (Per), GDP-6-deoxy-D-talose synthetase (GTS), GDP-Fucose synthetase Cys109Ser- (GFS-Cys109Ser) mutant, GDP-4-keto-6-deoxymannose-3-dehydratase (ColD), preferably GDP-4-keto-6-deoxymannose-3-dehydratase (ColD) in combination with GDP-L-colitose synthase (ColC), and variants thereof.

3. The vertebrate cell of claim 2, wherein the GDP-6-deoxy-D-lyxo-4-hexulose reductase (RMD) is from *Pseudomonas aeruginosa* (SEQ ID NO: 1).

4. The vertebrate cell of claims 1 to 3 further comprising GDP-D-rhamnose, GDP-D-perosamine, GDP-deoxy-D-talose, GDP-6-deoxy-D-altrose, GDP-4-keto-3,6-dideoxy-D-mannose, and/or GDP-L-colitose.

5. The vertebrate cell of claims 1 to 4, wherein the enzyme is expressed from a nucleic acid transiently present or stably maintained in said cell.

6. The vertebrate cell of claims 1 to 5, wherein the cell further comprises at least one molecule which is capable of being a substrate for a fucosyltransferase.

7. The vertebrate cell of claim 6, wherein the molecule is a protein or a lipid.

8. The vertebrate cell of claim 7, wherein the protein is an endogenous or an exogenous protein.

9. The vertebrate cell of claims 7 or 8, wherein the protein is an antibody, preferably an IgG1 antibody, an antibody fragment, preferably an antibody fragment comprising the Fc region of an antibody, a fusion protein, preferably a fusion protein comprising the Fc region of an antibody, a virus protein, virus protein fragment, an antigen, or a hormone.

10. The vertebrate cell of claims 1 to 9, wherein the vertebrate cell is a mammalian, a fish, an amphibian, a reptilian cell or an avian cell.

11. The vertebrate cell of claim 10, wherein

i) the mammalian cell is a human, hamster, canine or monkey cell, preferably a Chinese hamster ovary cell (CHO), an African green monkey kidney cell (VERO-76) (ATCC CRL-1587), a human cervical carcinoma cell (HELA) (ATCC CCL 2), a Madin Darbin canine kidney cell (MDCK) (ATCC CCL 34), a human PER.C6 cell, or a human AGE1.HN cell;

ii) the fish cell is a Ictalurus punctatus (channel catfish) ovary (CCO) cell (ATCC CRL-2772),

iii) the amphibian cell is a Xenopus laevis kidney cell (ATCC CCL-102);

iv) the reptilian cell is a Iguana iguana heart cells (IgH-2) (ATCC CCL-108); or

v) the avian cell is an avian retina cell, preferably a AGE1.CR.PIX cell, or an avian somite cell.

12. The vertebrate cell according to claims 1 to 11 which further comprises at least one glycosyltransferase for GDP-D-rhamnose, GDP-D-perosamine, GDP-deoxy-D-talose, GDP-6-deoxy-D-altrose, GDP-4-keto-3,6-dideoxy-D-mannose, or GDP-L-colitose.

13. A method for producing a molecule, which naturally comprises fucose on its glycomoieties, lacking fucose or with a reduced amount of fucose on its glycomoieties comprising the steps of:

i) providing a vertebrate cell according to claims 1 to 12,

ii) isolating the molecule which is capable of being a substrate for a fucosyltransferase, preferably a protein or lipid, from the cell in i).

14. A molecule lacking fucose or with a reduced amount of fucose on its glycomoieties obtainable by the method of claim 13.

15. A composition of molecules according to claim 14.

16. A composition comprising glycoproteins which comprise
i) between 70 and 95% of G0 -GlcNac, G0, G1, and/or G2 complex type N-glycans, and
ii) between 5 and 30% high mannose type N-glycans,
wherein the complex type N-glycans of the glycoproteins are free of fucose or substantially free of fucose.

17. The composition of claim 16, wherein of the complex type N-glycans 75% are G0 and 25% are G0 -GlcNac, G1 and/or G2 complex type N-glycans.

18. The composition of claim 16, wherein of the high mannose type N-glycans 50% are man5 glycans and 50% are man6, man7 and/or man8 N-glycans.

19. The composition of claims 16 to 18, wherein the glycoproteins are antibodies, preferably, IgG1s.

20. A protein or lipid which comprises glycomoieties containing D-rhamnose, D-perosamine, deoxy-D-talose, 6-deoxy-D-altrose, 4-keto-3,6-dideoxy-D-mannose, and/or L-colitose.

21. An expression unit comprising one or more vertebrate expression control sequences operably linked to a polynucleotide comprising a nucleic acid sequence encoding an enzyme which uses GDP-6-deoxy-D-lyxo-4-hexulose as a substrate, wherein the enzyme does not catalyze the reaction which converts GDP-6-deoxy-D-lyxo-4-hexulose into GDP-L-fucose.

22. The expression unit of claim 21, wherein the enzyme which uses GDP-6-deoxy-D-lyxo-4-hexulose as a substrate is selected from the group consisting of GDP-6-deoxy-D-lyxo-4-hexulose reductase (RMD), GDP-perosamine synthetase (Per), GDP-6-deoxy-D-talose synthetase (GTS), GDP-Fucose synthetase Cys109Ser- (GFS-Cys109Ser) mutant, GDP-4-keto-6-deoxymannose-3-dehydratase (ColD), preferably GDP-4-keto-6-deoxymannose-3-dehydratase (ColD) in combination with GDP-L-colitose synthase (ColC), and variants thereof.

23. An eukaryotic cell for producing a protein, which normally comprises fucose on its glycomoieties, lacking fucose or with a reduced amount of fucose on its glycomoieties comprising:

i) a first polynucleotide comprising a nucleic acid sequence encoding an enzyme which uses GDP-6-deoxy-D-lyxo-4-hexulose as a substrate, and

ii) a second polynucleotide comprising a nucleic acid sequence encoding a protein,

wherein the enzyme does not catalyze the reaction which converts GDP-6-deoxy-D-lyxo-4-hexulose into GDP-L-fucose.

24. The eukaryotic cell of claim 23, wherein the enzyme which uses GDP-6-deoxy-D-lyxo-4-hexulose as a substrate is selected from the group consisting of GDP-6-deoxy-D-lyxo-4-hexulose reductase (RMD), GDP-perosamine synthetase (Per), GDP-6-deoxy-D-talose synthetase (GTS), GDP-Fucose synthetase Cys109Ser- (GFS-Cys109Ser) mutant, GDP-4-keto-6-deoxymannose-3-dehydratase (ColD), preferably GDP-4-keto-6-deoxymannose-3-dehydratase (ColD) in combination with GDP-L-colitose synthase (ColC), and variants thereof.

25. The eukaryotic cell of claims 23 or 24, wherein the protein is an antibody, preferably an IgG1 antibody, an antibody fragment, preferably an antibody fragment comprising the Fc region of an antibody, a fusion protein comprising the Fc region of an antibody, a virus protein, virus protein fragment, an antigen, or a hormone.

26. A method for producing a protein, which normally comprises fucose on its glycomoieties, lacking fucose or with a reduced amount of fucose on its glycomoieties comprising the steps of:

i) providing an eukaryotic cell according to claims 23 to 25,

ii) expressing the enzyme encoded by the first polynucleotide and the protein encoded by the second polynucleotide in said cell, and

iii) isolating the protein from said cell.

27. A protein lacking fucose or with a reduced amount of fucose on its glycomoieties obtainable by the method of claim 26.

CLAIMS

1. A recombinant vertebrate cell for producing a protein molecule, lacking fucose or with a reduced amount of fucose on its glycomoieties, said cell comprising a polynucleotide that encodes at least one enzyme which uses GDP-6-deoxy-D-lyxo-4-hexulose as a substrate, wherein the enzyme does not catalyze the reaction which converts GDP-6-deoxy-D-lyxo-4-hexulose into GDP-L-fucose.

2. The vertebrate cell of claim 1, wherein the enzyme which uses GDP-6-deoxy-D-lyxo-4-hexulose as a substrate is selected from the group consisting of GDP-6-deoxy-D-lyxo-4-hexulose reductase (RMD), GDP-perosamine synthetase (Per), GDP-6-deoxy-D-talose synthetase (GTS), GDP-Fucose synthetase Cys109Ser-(GFS-Cys109Ser) mutant, GDP-4-keto-6-deoxymannose-3-dehydratase (ColD), and GDP-4-keto-6-deoxymannose-3-dehydratase (ColD) in combination with GDP-L-colitose synthase (ColC).

3. The vertebrate cell of claim 2, wherein the GDP-6-deoxy-D-lyxo-4-hexulose reductase (RMD) is from Pseudomonas aeruginosa (SEQ ID NO: 1).

4. The vertebrate cell of claim 1 further comprising GDP-D-rhamnose, GDP-D-perosamine, GDP-deoxy-D-talose, GDP-6-deoxy-D-altrose, GDP-4-keto-3,6-dideoxy-D-mannose, and/or GDP-L-colitose.

5. The vertebrate cell of claim 1, wherein the polynucleotide is transiently present or stably maintained in said cell.

6. The vertebrate cell of claim 1, wherein the cell comprises said protein molecule which is capable of being a substrate for a fucosyltransferase.

7. The vertebrate cell of claim 1, wherein the protein molecule is an endogenous or an exogenous protein.

8. The vertebrate cell of claim 1, wherein the protein molecule is an antibody, an antibody fragment, a fusion protein, a virus protein, a virus protein fragment, an antigen, or a hormone.

9. The vertebrate cell of claim 1, wherein the vertebrate cell is a mammalian, a fish, an amphibian, a reptilian cell or an avian cell.

10. The vertebrate cell of claim 9, wherein

i) the mammalian cell is a human, hamster, canine or monkey cell;

ii) the fish cell is a Ictalurus punctatus (channel catfish) ovary (CCO) cell;

iii) the amphibian cell is a Xenopus laevis kidney cell;

iv) the reptilian cell is a Iguana iguana heart cell (IgH-2); or

v) the avian cell is an avian retina cell, or an avian somite cell.

11. The vertebrate cell according to claim 1 which further comprises at least one glycosyltransferase for GDP-D-rhamnose, GDP-D-perosamine, GDP-deoxy-D-talose, GDP-6-deoxy-D-altrose, GDP-4-keto-3,6-dideoxy-D-mannose, or GDP-L-colitose.

12. A method for producing a protein molecule, lacking fucose or with a reduced amount of fucose on its glycomoieties comprising the steps of:

i) providing a vertebrate cell according to claim 1,

ii) isolating the protein molecule which is capable of being a substrate for a fucosyltransferase from the cell in i).

13. A protein molecule lacking fucose or with a reduced amount of fucose on its glycomoieties obtainable by the method of claim 12.

14. A composition of protein molecules according to claim 13.

15. A protein molecule which comprises glycomoieties containing D-rhamnose, D-perosamine, deoxy-D-talose, 6-deoxy-D-altrose, 4-keto-3,6-dideoxy-D-mannose, and/or L-colitose, wherein said protein molecule is obtainable by the method of claim 12.

16. An expression unit comprising one or more vertebrate expression control sequences operably linked to a polynucleotide comprising a nucleic acid sequence encoding an enzyme which uses GDP-6-deoxy-D-lyxo-4-hexulose as a substrate, wherein the enzyme does not catalyze the reaction which converts GDP-6-deoxy-D-lyxo-4-hexulose into GDP-L-fucose.

17. The expression unit of claim 16, wherein the enzyme which uses GDP-6-deoxy-D-lyxo-4-hexulose as a substrate is selected from the group consisting of GDP-6-deoxy-D-lyxo-4-hexulose reductase (RMD), GDP-perosamine synthetase (Per), GDP-6-deoxy-D-talose

synthetase (GTS), GDP-Fucose synthetase Cys109Ser-(GFS-Cys109Ser) mutant, GDP-4-keto-6-deoxymannose-3-dehydratase (ColD), and GDP-4-keto-6-deoxymannose-3-dehydratase (ColD) in combination with GDP-L-colitosc synthase (ColC).

18. A recombinant eukaryotic cell for producing a protein, lacking fucose or with a reduced amount of fucose on its glycomoieties comprising:

i) a first polynucleotide comprising a nucleic acid sequence encoding an enzyme which uses GDP-6-deoxy-D-lyxo-4-hexulose as a substrate, and

ii) a second polynucleotide comprising a nucleic acid sequence encoding a protein, wherein the enzyme does not catalyze the reaction which converts GDP-6-deoxy-D-lyxo-4-hexulose into GDP-L-fucose.

19. The eukaryotic cell of claim 18, wherein the enzyme which uses GDP-6-deoxy-D-lyxo-4-hexulose as a substrate is selected from the group consisting of GDP-6-deoxy-D-lyxo-4-hexulose reductase (RMD), GDP-perosamine synthetase (Per), GDP-6-deoxy-D-talose synthetase (GTS), GDP-Fucose synthetase Cys109Ser-(GFS-Cys109Ser) mutant, GDP-4-keto-6-deoxymannose-3-dehydratase (ColD), and GDP-4-keto-6-deoxymannose-3-dehydratase (ColD) in combination with GDP-L-colitose synthase (ColC).

20. The eukaryotic cell of claim 18, wherein the protein is an antibody, an antibody fragment, a fusion protein comprising the Fc region of an antibody, a virus protein, a virus protein fragment, an antigen, or a hormone.

21. A method for producing a protein lacking fucose or with a reduced amount of fucose on its glycomoieties comprising the steps of:

i) providing an eukaryotic cell according to claim 18,

ii) expressing the enzyme encoded by the first polynucleotide and the protein encoded by the second polynucleotide in said cell, and

iii) isolating the protein from said cell.

22. A protein lacking fucose or with a reduced amount of fucose on its glycomoieties obtainable by the method of claim 21.

23. The vertebrate cell of claim 7, wherein the protein molecule is an antibody, an antibody fragment, a fusion protein, a virus protein, a virus protein fragment, an antigen, or a hormone.