mannose glycoform content by manipulating levels of expression of proteins involved in the N-glycosylation pathway.

SUMMARY

The present disclosure provides a method for regulating the high mannose glycoform content of a recombinant protein during a mammalian cell culture process comprising transforming a host cell to overexpress a protein that is involved in the N-glycosylation pathway. The disclosure further provides a method for decreasing the high mannose glycoform content of a recombinant protein during the mammalian cell culture process. In one example, the protein is N-acetyl-glucosaminyltransferase-1 (encoded by Mgat1); in another example of the disclosure, the protein is N-acetyl-glucosaminyltransferase-2 (encoded by Mgat2). In a further example of the disclosure, the protein is a UDP-Galactose transporter (encoded by Slc35a2).

In one aspect, the present disclosure refers to a method of decreasing the high mannose glycoform content of a recombinant protein during a mammalian cell culture process, compared to a culture in which mammalian cells are not subject to a transfection to overexpress proteins involved in an N-glycosylation pathway, the method comprising: transfecting a mammalian host cell to overexpress a protein that is involved in an N-glycosylation pathway, wherein the protein involved in the N-glycosylation pathway is selected from the group consisting of N-acetyl-glucosaminyltransferase-1 encoded by Mgat1; N-acetyl-glucosaminyltransferase-2 encoded by Mgat2; a UDP-Galactose transporter encoded by Slc35a2; and combinations thereof; and either (a) subjecting the transfected mammalian host cells to a temperature shift; or (b) inducing the transfected mammalian host cells into cell growth arrest by L-asparagine starvation followed by perfusion with a serum-free perfusion media having an L-asparagine concentration of 5 mM or less, whereby said high mannose glycoform content of the recombinant protein is decreased.

Additional example of the disclosure include transformation of the host cell to overexpress two or more proteins involved in the N-glycosylation pathway, including combinations of the aforementioned proteins. In one example, the host cell is transformed with Mgat1 and Mgat2; in other example, the host cell is transformed with Mgat1 and Slc35a2, with Mgat2 and Slc35a2, or with Mgat1, Mgat2 and Slc35a2.

The disclosure also provides for transfection of a host cell line that has been previously transfected to express a recombinant protein. In one example, the recombinant protein is a protein comprising an antibody Fc region. Further example includes host cells that express a recombinant protein selected from the group consisting of Fc fusion proteins, antibodies, immunoglobulins, and peptibodies.

CLAIMS

What is claimed is:

1. A method of decreasing the high mannose glycoform content of a recombinant protein during a mammalian cell culture process, compared to a culture in which mammalian cells are not subject to a transfection to overexpress proteins involved in an N-glycosylation pathway, the method comprising:

transfecting a mammalian host cell to overexpress a protein that is involved in an N-glycosylation pathway, wherein the protein involved in the N-glycosylation pathway is selected from the group consisting of N-acetyl-glucosaminyltransferase-1 encoded by Mgat1; N-acetyl-glucosaminyltransferase-2 encoded by Mgat2; a UDP-Galactose transporter encoded by Slc35a2; and combinations thereof; and either

(a) subjecting the transfected mammalian host cells to a temperature shift; or

(b) inducing the transfected mammalian host cells into cell growth arrest by L-asparagine starvation followed by perfusion with a serum-free perfusion media having an L-asparagine concentration of 5 mM or less,

whereby said high mannose glycoform content of the recombinant protein is decreased.

2. The method of claim 1, wherein the temperature shift is from 36 ºC to 31 ºC, or from 36 ºC to 33 ºC.

3. The method of claim 1, wherein the mammalian cell culture process comprises a growth phase and a production phase, wherein the temperature shift occurs at a transition between the growth phase and production phase, or during the production phase.

4. The method of claim 1, wherein the perfusion media has an L-asparagine concentration selected from the group consisting of:

(a) 4.0 mM or less;

(b) 3.0 mM or less;

(c) 2.0 mM or less;

(d) 1.0 mM or less; and

(e) 0 mM.

5. The method according to claim 1, wherein the host cell is transfected to express two or more proteins involved in the N-glycosylation pathway, wherein the proteins are selected from the group consisting of N-acetyl-glucosaminyltransferase-1 encoded by Mgat1 and N-acetyl-glucosaminyltransferase-1 encoded by Mgat2; N-acetyl-

glucosaminyltransferase-1 encoded by Mgat1 and the UDP-Galactose transporter encoded by Slc35a2; N-acetyl-glucosaminyltransferase-1 encoded by Mgat2 and the UDP-Galactose transporter encoded by Slc35a2; and N-acetyl-glucosaminyltransferase-1 encoded by Mgat1, N-acetyl-glucosaminyltransferase-1 encoded by Mgat2 and the UDP-Galactose transporter encoded by Slc35a2.

6. The method of claim 5, wherein the mammalian host cell is transfected to express Mgat1 and Mgat2.

7. The method of claim 1, wherein the mammalian host cell line has been previously transfected to express the recombinant protein and is subsequently transfected to express a protein that is involved in an N-glycosylation pathway.

8. The method of claim 1, wherein the mammalian host cell line is first transfected with a protein that is involved in an N-glycosylation pathway and is subsequently transfected to express the recombinant protein.

9. The method of claim 1, wherein the recombinant protein is selected from the group consisting of a protein comprising an antibody Fc region, a Fc fusion protein, an antibody, an immunoglobulin, and a peptibody.

10. The method of claim 1, wherein the high mannose glycoform content of the recombinant protein expressed in the mammalian host cells that have been transfected to overexpress a protein that is involved in an N-glycosylation pathway is decreased compared to that produced by the mammalian host cell before being transfected to overexpress a protein involved in an N-glycosylation pathway.

11. The method of claim 1, wherein the high mannose glycoform content of the recombinant protein is less than or equal to 10%.

12. The method of claim 1, wherein the high mannose glycoform content of the recombinant protein is less than or equal to 5%.

13. The method of claim 1, wherein the mammalian cell culture is a perfusion culture.

14. The method according to claim 13, wherein the mammalian cell culture is perfused using alternating tangential flow (ATF).

15. The method of claim 13, wherein perfusion begins on or about day 1 to on or about day 9 of the mammalian cell culture, or wherein perfusion begins on or about day 3 to on or about day 7, of the mammalian cell culture.

16. The method of claim 13, wherein perfusion begins when the transfected mammalian cells have reached a production phase.

17. The method of claim 13, wherein the mammalian cell culture is maintained by fed batch.

18. The method of claim 17, wherein the mammalian cell culture is fed three times or four times during production.

19. The method of claim 1, wherein the mammalian host cell is a Chinese Hamster Ovary (CHO) cell.

20. The method of claim 1, wherein the method further comprises a harvest step for the recombinant protein.

21. The method of claim 20, wherein the harvested recombinant protein is purified and formulated in a pharmaceutically acceptable formulation.