WO 2019/126634 disclosed targeted integration (TI) host cells suitable for the expression of recombinant proteins, as well as methods of producing and using said TI host cells.

Magistrelli et al. (J. Immunol. Meth. 375 (2012) 20-29) reported a method for producing biotinylated FcRn using PEAK cells stably expressing EBNA-1 and transiently transfected with a dual promoter vector for FcRnα and β2m co-expression (i) the biotin ligase enzyme (LsBirA) and (ii) the enhanced green fluorescent protein (EGFP) as a reporter gene. After Ni-NTA purification and immobilization they obtained only 4 mg/L and 1.2 mg/L, respectively, per liter of cell culture supernatant for hFcRn and mFcRn.

Farber-Schwarz, A., reported serum albumin and its interaction with the neonatal Fc Receptor - Characterization of the albumin/FcRn-binding mechanism (Doctoral Thesis, Univesity of Stuttgart, Germany, 2013). In this work a soluble form of the human FcRn was generated using the bicistronic Lonza pEE 6.4 expression vector. The production of a soluble mouse FcRn using the Lonza expression system was not possible. No yields are disclosed.

Summary

Herein is reported a recombinant mammalian cell expressing neonatal Fc receptor (FcRn). FcRn is a heteromultimeric polypeptide not naturally expressed by said mammalian cell. More specifically, FcRn is a heterodimeric protein consisting of two polypeptides: a class I major histocompatibility complex-like protein (α-FcRn) and β2-microglobulin (β2m). To achieve expression of FcRn a recombinant nucleic acid comprising multiple different expression cassettes in a specific and defined sequence has been integrated into the genome of a mammalian cell.

Herein is also reported a method for generating a recombinant mammalian cell expressing FcRn and a method for producing FcRn using said recombinant mammalian cell.

The current disclosure is based, at least in part, on the finding that the sequence of the different expression cassettes required for the expression of the heterodimeric FcRn, i.e. the expression cassette organization, as integrated into the genome of a mammalian cell influences the expression yield of FcRn.

The current disclosure is based, at least in part, on the finding that by integrating a nucleic acid encoding the heterodimeric FcRn that has a specific expression cassette organization into the genome of a mammalian cell efficient recombinant expression and production of FcRn can be achieved.

It has been found that the defined expression cassette sequence can advantageously be integrated into the genome of a mammalian cell by a double recombinase mediated cassette exchange reaction.

In one aspect, the present disclosure provides a method for producing C-terminally biotinylated FcRn comprising the steps of

a) cultivating a mammalian cell comprising a deoxyribonucleic acid encoding FcRn and biotin-[acetyl-CoA-carboxylase] ligase (BirA) in a biotin containing medium, and

b) recovering C-terminally biotinylated FcRn from the cell or the cultivation medium,

wherein the deoxyribonucleic acid encoding FcRn and BirA is stably integrated into the genome of the mammalian cell and comprises in 5'- to 3'-direction

- a first expression cassette encoding class I major histocompatibility complex-like protein (α-FcRn) comprising an Avi-tag at the C-terminus,
- a second expression cassette encoding β2-microglobulin (β2m),
- a third expression cassette encoding class I major histocompatibility complex-like protein (α-FcRn) comprising an Avi-tag at the C-terminus,
- a fourth expression cassette encoding β2-microglobulin (β2m), and
- a fifth expression cassette encoding biotin-[acetyl-CoA-carboxylase] ligase.

One example of the current disclosure is a method for producing C-terminally biotinylated FcRn comprising the steps of

a) cultivating a mammalian cell comprising a deoxyribonucleic acid encoding FcRn and E.coli biotin-[acetyl-CoA-carboxylase] ligase (BirA) in a biotin containing medium, and

b) recovering C-terminally biotinylated FcRn from the cell or the cultivation medium,

wherein the deoxyribonucleic acid encoding FcRn and E.coli BirA is stably integrated into the genome of the mammalian cell and comprises in 5'- to 3'-direction

- a first expression cassette encoding class I major histocompatibility complex-like protein (α-FcRn) comprising a HisAvi-tag at the C-terminus,
- a second expression cassette encoding β2-microglobulin (β2m),
- a third expression cassette encoding class I major histocompatibility complex-like protein (α-FcRn) comprising a HisAvi-tag at the C-terminus,
- a fourth expression cassette encoding β2-microglobulin (β2m), and
- a fifth expression cassette encoding E.coli biotin-[acetyl-CoA-carboxylase] ligase.

In one example the deoxyribonucleic acid encoding FcRn and E.coli BirA further comprises

- a first recombination recognition sequence located 5' to the first expression cassette,
- a second recombination recognition sequence located 3' to the fifth expression cassette, and
- a third recombination recognition sequence located between the second and the third expression cassette,

and

wherein all recombination recognition sequences are different.

In one example the deoxyribonucleic acid encoding FcRn and BirA comprises a further expression cassette encoding for a selection marker and the expression cassette encoding for the selection marker is located partly 5' and partly 3' to the third recombination recognition sequences, wherein the 5'-located part of said expression cassette comprises the promoter and the start-codon and the 3'-located part of said expression cassette comprises the coding sequence without a start-codon and a polyA signal, wherein the start-codon is operably linked to the coding sequence.

In one example each of the expression cassettes comprise in 5'-to-3' direction a promoter, a coding sequence and a polyadenylation signal sequence optionally followed by a terminator sequence. In one example the promoter is the human CMV promoter with intron A, the polyadenylation signal sequence is the bGH polyA site and the terminator sequence is the hGT terminator except for the expression cassette of the selection marker, wherein the promoter is the SV40 promoter and the polyadenylation signal sequence site is the SV40 polyA site and a terminator sequence is absent.

In one example the mammalian cell is a CHO cell.

In one example the FcRn is human FcRn, the class I major histocompatibility complex-like protein (α-FcRn) is human class I major histocompatibility complex-like protein (α-FcRn), and the β2-microglobulin (β2m) is human β2-microglobulin (β2m).

In one example the FcRn is murine FcRn, the class I major histocompatibility complex-like protein (α-FcRn) is murine class I major histocompatibility complex-like protein (α-FcRn), and the β2-microglobulin (β2m) is murine β2-microglobulin (β2m).

In one example the FcRn is cynomolgus FcRn, the class I major histocompatibility complex-like protein (α-FcRn) is cynomolgus class I major histocompatibility complex-like protein (α-FcRn), and the β2-microglobulin (β2m) is cynomolgus β2-microglobulin (β2m).

The current disclosure provides a biotinylated FcRn according to the disclosure.

The current disclosure provides the use of the biotinylated FcRn according to the disclosure as affinity chromatography ligand.

Detailed Description of Embodiments of the Invention

The current invention is based, at least in part, on the finding that for the expression of the neonatal Fc-receptor (FcRn), a complex molecule comprising different polypeptides, i.e. which is a heteromultimer, the use of a defined and specific expression cassette organization results in efficient expression and production of FcRn in mammalian cells, such as CHO cells.

The current invention is based, at least in part, on the finding that double recombinase mediated cassette exchange (RMCE) can be used for producing a recombinant mammalian cell, such as a recombinant CHO cell, in which a defined and specific expression cassette sequence has been integrated into the genome, which in turn results in the efficient expression and production of FcRn. The integration is effected at a specific site in the genome of the mammalian cell by targeted integration. Thereby it is possible to control the expression ratio of the different polypeptides of the heteromultimeric FcRn relative to each other. Thereby in turn an efficient expression, correct assembly and successful secretion in high expression yield of correctly folded and assembled FcRn is achieved.

I. DEFINITIONS

Useful methods and techniques for carrying out the current invention are described in e.g. Ausubel, F.M. (ed.), Current Protocols in Molecular Biology, Volumes I to III (1997); Glover, N.D., and Hames, B.D., ed., DNA Cloning: A Practical Approach, Volumes I and II (1985), Oxford University Press; Freshney, R.I. (ed.), Animal Cell Culture – a practical approach, IRL Press Limited (1986); Watson, J.D., et al., Recombinant DNA, Second Edition, CHSL Press (1992); Winnacker, E.L., From Genes to Clones; N.Y., VCH Publishers (1987); Celis, J., ed., Cell Biology, Second Edition, Academic Press (1998); Freshney, R.I., Culture of Animal Cells: A Manual of Basic Technique, second edition, Alan R. Liss, Inc., N.Y. (1987).

The use of recombinant DNA technology enables the generation of derivatives of a nucleic acid. Such derivatives can, for example, be modified in individual or several