Klose; R.J. and Bird, A.P., Trends Biochem Sci 31 (2006): 89-97). Methylated promoters are in general inactive.

The very strong promoter and enhancer of the major immediate-early genes of the human cytomegalovirus (hCMV-MIE) is used for the recombinant expression of polypeptides in mammalian cells. It has been shown that the promoter is prone to silencing by methylation in transient as well as stably transfected mammalian cells(see e.g. Escher, G., et al. J. Lipid Res. 46 (2005) 356-365; Krishnan, M., et al., FASEB J. 20 (2006) 106-108; Proesch, S. et al., Biol Chem Heppe Seyler 377 (1996): 195-201; Yang, Y., et al., J. Biotechnol. 147 (2010) 180-185).

It has been demonstrated previously in WO 2011/128377 that direct methylation of the hCMV-MIE promoter can be used as early marker to predict production instability of recombinant CHO cell lines.

Osterlehner, A., et al., report promoter methylation and trans gene copy numbers predict unstable protein production in recombinant Chinese hamster ovary cell lines and describe that the different CpG sites are methylated with different frequency (Biotechnol. Bioeng. 108 (2011) 2670-2681).

Summary

It has been found that a C to G point mutation, i.e. a single C to G mutation, in the hCMV-MIE promoter at specific sites results in reduced promoter silencing and likewise in improved production stability. In addition, it has been found that higher titers of produced polypeptide can be achieved. Mutation of C to G at position -41 and/or at position -179 relative to the transcription start site have shown to be particularly effective.

As reported herein is a human CMV promoter (based on the promoter of SEQ ID NO: 01) that has at nucleotide position -41 (and/) or -179 relative to the transcription start site the nucleotide G.

In one embodiment the human CMV promoter has improved production stability and/or improved product titer (when compared to human CMV promoters without C to G point mutations at position -41 and/or -179 relative to the transcriptions start site).

In one embodiment the human CMV promoter has the nucleic acid sequence of SEQ ID NO: 02 or SEQ ID NO: 03.

One aspect as reported herein is a promoter that has the nucleic acid sequence of SEQ ID NO: 02 or SEQ ID NO: 03.

One aspect as reported herein is a method for the production of a polypeptide, characterized in that it comprises the following steps:
a) transfecting a eukaryotic cell with a nucleic acid comprising an expression cassette comprising a first nucleic acid of SEQ ID NO: 02 or SEQ ID NO: 03 operably linked to a second nucleic acid encoding the polypeptide,
b) selecting a cell transfected in step a),
c) cultivating the selected cell of step b),
d) recovering the polypeptide from the cell or the cultivation medium,
and thereby producing the polypeptide.
One aspect as reported herein is use of a promoter that has the nucleic acid sequence of SEQ ID NO: 02 or SEQ ID NO: 03 for the production of a polypeptide.

One aspect as reported herein is a cell comprising the promoter as defined herein.

As reported herein is a promoter that has the nucleic acid sequence of SEQ ID NO: 02.

As reported herein is a nucleic acid characterized in that it is consisting of the nucleic acid of SEQ ID NO: 02 and has a promoter strength of at least 80% of the human CMV major immediate-early promoter of SEQ ID NO: 01 when operably linked to the nucleic acid of SEQ ID NO: 04 encoding the enhanced green fluorescent protein (eGFP).

As reported herein is a method for the production of a polypeptide, characterized in that it comprises the following steps:

a) transfecting a eukaryotic cell with a nucleic acid comprising an expression cassette comprising a first nucleic acid of SEQ ID NO: 02 operably linked to a second nucleic acid encoding the polypeptide,

b) selecting a cell transfected in step a),

c) cultivating the selected cell of step b) (under conditions suitable for the expression of the polypeptide),

d) recovering the polypeptide from the cell or the cultivation medium,

and thereby producing the polypeptide.

In one embodiment the production is a production at large scale. In one embodiment the production is at a final cultivation volume of 500 l or more, in one embodiment of 500 l to 10,000 l.

In one embodiment said polypeptide is an immunoglobulin, or an immunoglobulin-fragment, or an immunoglobulin-conjugate.

In one embodiment the polypeptide is an immunoglobulin light chain or an immunoglobulin heavy chain or a variant thereof or a fragment thereof or a fusion thereof.

In one embodiment the nucleic acid comprises a further expression cassette encoding a selectable marker. In one embodiment the nucleic

acid comprises a further expression cassette encoding a immunoglobulin light chain or an immunoglobulin heavy chain.

In one embodiment said eukaryotic cell is a mammalian cell.

In one embodiment said mammalian cell is a CHO cell, a BHK cell, a HEK cell, a Sp2/0 cell or a Per.C6® cell.

In one embodiment said mammalian cell is a CHO cell or a HEK cell.

In one embodiment said selectable marker is a dihydrofolate reductase, an aminoglycoside phosphotransferase or a hygromycin-phosphotransferase.

As reported herein is the use of a promoter of SEQ ID NO: 02 for the production of a polypeptide.

As reported herein is a cell comprising the promoter as reported herein or the nucleic acid as reported herein.

As reported herein is a promoter that has the nucleic acid sequence of SEQ ID NO: 03

As reported herein is a nucleic acid characterized in that it is consisting of the nucleic acid of SEQ ID NO: 03 and has a promoter strength of at least 80% of the human CMV major immediate-early promoter of SEQ ID NO: 01 when operably linked to the nucleic acid of SEQ ID NO: 04 encoding the enhanced green fluorescent protein (eGFP).

As reported herein is a method for the production of a polypeptide, characterized in that it comprises the following steps:

a) transfecting a eukaryotic cell with a nucleic acid comprising an expression cassette comprising a first nucleic acid of SEQ ID NO: 03 operably linked to a second nucleic acid encoding the polypeptide,

b) selecting a cell transfected in step a),

c) cultivating the selected cell of step b) (under conditions suitable for the expression of the polypeptide),

d) recovering the polypeptide from the cell or the cultivation medium,

and thereby producing the polypeptide.

In one embodiment the production is a production at large scale. In one embodiment the production is at a final cultivation volume of 500 l or more, in one embodiment of 500 l to 10,000 l.

In one embodiment said polypeptide is an immunoglobulin, or an immunoglobulin-fragment, or an immunoglobulin-conjugate.

In one embodiment the polypeptide is an immunoglobulin light chain or an immunoglobulin heavy chain or a variant thereof or a fragment thereof or a fusion thereof.

In one embodiment the nucleic acid comprises a further expression cassette encoding a selectable marker. In one embodiment the nucleic acid comprises a further expression cassette encoding a immunoglobulin light chain or an immunoglobulin heavy chain.

In one embodiment said eukaryotic cell is a mammalian cell.

In one embodiment said mammalian cell is a CHO cell, a BHK cell, a HEK cell, a Sp2/0 cell or a Per.C6® cell.

In one embodiment said mammalian cell is a CHO cell or a HEK cell.

In one embodiment said selectable marker is a dihydrofolate reductase, an aminoglycoside phosphotransferase or a hygromycin-phosphotransferase.

As reported herein is the use of a promoter of SEQ ID NO: 03 for the production of a polypeptide.

As reported herein is a cell comprising the promoter as reported herein or the nucleic acid as reported herein.

As reported herein is a human CMV promoter that has at nucleotide position -41 relative to the transcription start site the nucleotide G.

In one embodiment the human CMV promoter is a human CMV major immediate-early promoter.

As reported herein is a human CMV promoter that has at nucleotide position -179 relative to the transcription start site the nucleotide G.

In one embodiment the human CMV promoter is a human CMV major immediate-early promoter.

Detailed Description of the Invention

When using stably transfected eukaryotic cell lines for the production/expression of recombinant polypeptides, like secreted proteins, an intracellular reporterprotein or a cell surface marker, the gradual loss of productivity in long-term culture is a common issue with the development of manufacturing cell lines (Barnes, L.M., et al. Biotechnol. Bioeng. 81 (2003) 631-639). For production cell lines/cell clones, i.e. cell clone/cell lines that are to be used for the large scale recombinant production of a polypeptide, such as e.g. an antibody, the production stability, i.e. the loss of productivity generation by generation, of the cell clone/cell line is important. Thus, mammalian cell lines for recombinant protein production need to maintain productivity over extended cultivation times. Generally the production stability of a cell clone/cell line is determined by cultivating the cell clone/cell line over a long period of time. At regular intervals the medium is diluted with fresh medium and the specific productivity per cell is determined based on the product titer and the viable cell density. The change (normally a reduction) of the specific productivity is indicative of long term production stability of the cell clone/cell line.

The decrease of recombinant protein expression can be due to a loss of transgene copies and/or silencing of the transgene promoter (see e.g. Escher, G., et al. J. Lipid Res. 46 (2005) 356-365; Krishnan, M., et al., FASEB J. 20 (2006) 106-108; Yang, Y., et al., J. Biotechnol. 147 (2010) 180-185). Silencing of promoters is caused by epigenetic modifications of chromatin such as posttranslational modifications of histones as well as direct methylation of promoter DNA at CpG sites (see e.g. Cedar, H. and Bergman, Y., Nat. Rev. Genet. 10 (2009) 295-304; De Carvalho, D.D. et al., Trends Cell Biol 20 (2010): 609-617; Klose; R.J. and Bird, A.P., Trends Biochem Sci 31 (2006): 89-97). Methylated promoters are in general inactive.

Patent Claims

1. Promoter that has the nucleic acid sequence of SEQ ID NO: 02 or SEQ ID NO: 03.

2. The promoter according to claim 1, wherein said promoter has a promoter strength of at least 80% of the human CMV-major immediate-early promoter of SEQ ID NO: 01 when operably linked to the nucleic acid of SEQ ID NO: 04.

3. Method for the production of a polypeptide, characterized in that it comprises the following steps:

   a) transfecting a eukaryotic cell with a nucleic acid comprising an expression cassette comprising a first nucleic acid of SEQ ID NO: 02 or SEQ ID NO: 03 operably linked to a second nucleic acid encoding the polypeptide,
   b) selecting a cell transfected in step a),
   c) cultivating the selected cell of step b),
   d) recovering the polypeptide from the cell or the cultivation medium,

   and thereby producing the polypeptide.

4. Method according to claim 3 wherein the production is a production at large scale.

5. Method according to any one of claims 3 to 4 wherein the polypeptide is an immunoglobulin light chain or an immunoglobulin heavy chain or a variant thereof or a fragment thereof or a fusion thereof.

6. Method according to any one of claims 3 to 5, characterized in that the nucleic acid comprises a further expression cassette encoding a selectable marker.

7. Method according to any one of claims 3 to 6, characterized in that said eukaryotic cell is a mammalian cell.

8. Method according to claim 7, characterized in that said mammalian cell is a CHO cell, a BHK cell, a HEK cell or a Sp2/0 cell.

9. Method according to claim 8, characterized in that said mammalian cell is a CHO cell or a HEK cell.

10. Method according to any one of claims 3 to 9, characterized in that said polypeptide is an immunoglobulin, or an immunoglobulin-fragment, or an immunoglobulin-conjugate.

11. Method according to any one of claims 7 to 10, characterized in that said selectable marker is a dihydrofolate reductase, an aminoglycoside phosphotransferase or a hygromycin-phosphotransferase.

12. Use of a promoter that has the nucleic acid sequence of SEQ ID NO: 02 or SEQ ID NO: 03 for the production of a polypeptide.

13. A cell comprising the promoter according to claim 1 or claim 2.