One aspect as reported herein is a method for the recombinant production of a polypeptide in a eukaryotic cell comprising the steps of:

- cultivating a eukaryotic cell comprising a nucleic acid encoding the polypeptide in a cultivation medium comprising a compound selected from the group consisting of trans-2-methyl 2-pentenoic acid, the broad-spectrum histone deacetylase (HDAC) inhibitor Quisinostat, and the subtype-specific HDAC inhibitor Romidepsin, and
- recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide in a eukaryotic cell.

In one embodiment 2M2P or Quisinostat or Romidepsin is added of from one hour to 24 hours after the after transfection of the cells to the cultivation. In one embodiment 2M2P or Quisinostat or Romidepsin is added about three hours after the transfection of the cells to the cultivation. Thus, in one embodiment the

cultivating is in a cultivation medium to which 2M2P or Quisinostat or Romidepsin has been added after the transfection.

In one embodiment 2M2P (trans-2-methyl 2-pentenoic acid) is added to a final concentration in the cultivation medium of about 6-7 mM (at least 3 mM).

As reported herein is a method for the recombinant production of a polypeptide in a eukaryotic cell comprising the steps of:

- cultivating a eukaryotic cell comprising a nucleic acid encoding the polypeptide in a cultivation medium comprising an atypical/non-canonical NFκB activator, and
- recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide in a eukaryotic cell.

In one embodiment the non-canonical NFκB activator is selected from the group consisting of EGF, PMA, and betulinic acid.

As reported herein is a method for the recombinant production of a polypeptide in a eukaryotic cell comprising the steps of:

- cultivating a eukaryotic cell comprising a nucleic acid encoding the polypeptide in a cultivation medium comprising a first and a second compound, wherein said first compound is a subtype-specific HDAC inhibitor selected from the group consisting of an HDAC-1 inhibitor, an HDAC-2 inhibitor and an HDAC-3 inhibitor, and said second compound is a canonical or non-canonical nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) activator selected from the group consisting of Tumor necrosis factor (TNF) alpha, ligands of interleukin-1/Toll-like receptor (IL-1R/TLR), lipopolysaccharides, interleukin-1beta (IL-1b) epidermal growth factor (EGF), phorbol myristate acetate (PMA) and betulinic acid, and
- recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide in a eukaryotic cell.

In one embodiment the subtype-specific HDAC inhibitor is an HDAC-1 and/or HDAC-2 and/or HDAC-3 inhibitor. In one embodiment the subtype-specific HDAC inhibitor is selected from the group consisting of pimelic diphenylamide 106, Apicidin, and Romidepsin.

In the above methods different functional pathways are modified in order to increase transient gene expression yield. It has now been found that a further increase in transient gene expression yield is possible if production enhancers using different modes of action, i.e. modifying different functional pathways, are combined. The combination of production enhancer modifying the same functional

Patent Claims

1. A method for the recombinant production of a polypeptide in a eukaryotic cell comprising the steps of:

   - cultivating a eukaryotic cell comprising a nucleic acid encoding the polypeptide in a cultivation medium comprising a compound selected from the group consisting of trans-2-methyl 2-pentenoic acid, the broad-spectrum histone deacetylase (HDAC) inhibitor Quisinostat, and the subtype-specific HDAC inhibitor Romidepsin, and

   - recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide in a eukaryotic cell.

2. A method for the recombinant production of a polypeptide in a eukaryotic cell comprising the steps of:

   - cultivating a eukaryotic cell comprising a nucleic acid encoding the polypeptide in a cultivation medium comprising a first and a second compound, wherein said first compound is a subtype-specific HDAC inhibitor selected from the group consisting of an HDAC-1 inhibitor, an HDAC-2 inhibitor and an HDAC-3 inhibitor, and said second compound is a canonical or non-canonical nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) activator selected from the group consisting of Tumor necrosis factor (TNF) alpha, ligands of interleukin-1/Toll-like receptor (IL-1R/TLR), lipopolysaccharides, interleukin-1beta (IL-1b) epidermal growth factor (EGF), phorbol myristate acetate (PMA) and betulinic acid, and

   - recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide in a eukaryotic cell.

3. The method according to claim 2, wherein the subtype-specific HDAC inhibitor is selected from the group consisting of pimelic diphenylamide 106, Apicidin, and Romidepsin.

4. The method according to claim 2 or 3, wherein the first compound is trans-2-methyl 2-pentenoic acid and the second compound is Flagellin.

5. The method according to claim 2 or 3, wherein the first compound is trans-2-methyl 2-pentenoic acid and the second compound is Quisinostat.

6. The method according to claim 2 or 3, wherein the first compound is Romidepsin and the second compound is trans-2-methyl 2-pentenoic acid or Flagellin.

7. The method according to any one of claims 1 to 6, wherein the compound or the first compound is added of from one hour to five hours after transfection of the cells.

8. The method according to claim 7, wherein the compound or the first compound is added about three hours after the transfection of the cells to the cultivation.

9. The method according to any one of claims 2 to 8, wherein the second compound is added from twenty-one hours to thirty hours after the transfection of the cells to the cultivation.

10. The method according to claim 9, wherein the second compound is added about twenty-four to twenty-seven hours after the transfection of the cells to the cultivation.

11. The method according to any one of claims 1 to 10, wherein trans-2-methyl 2-pentenoic acid is added to a final concentration in the cultivation medium of about 6-7 mM.

12. The method according to any one of claims 6 to 10, wherein Flagellin is added to a final a concentration of 2 ng/mL and Romidepsin is added to a final concentration of 15 nM.

13. The method according to any one of claims 1 to 12, wherein the polypeptide is an antibody.

14. The method according to any one of claims 1 to 13, wherein the eukaryotic cell is a Chinese hamster ovary (CHO) cell or a human embryonic kidney (HEK) cell.