

CLAIMS

1. An ex vivo, in vitro or in cellulo method for expressing a recombinant DNA molecule in a eukaryotic host cell, comprising the steps of:
 - 5 (a) expressing or introducing at least one chimeric protein, in said host cell, wherein said chimeric protein comprises:
 - at least one catalytic domain of a capping enzyme, in particular selected in the group consisting of cap-0 canonical capping enzymes, cap-0 non-canonical capping enzymes, cap-1 capping enzymes and cap-2 capping enzymes; and
 - 10 ○ at least one catalytic domain of a DNA-dependent RNA polymerase, in particular a bacteriophage DNA-dependent RNA polymerase,
 - (b) constitutively or transiently downregulating the phosphorylation level of subunit α of translation initiation factor eIF2 (eIF2 α) in said host cell.
- 15 2. The method according to claim 1, wherein step (b) comprises introducing, into said host cell, at least one polypeptide or a nucleic acid molecule encoding said polypeptide, wherein the polypeptide modulates the activity or the expression of a target host cell protein involved in the regulation of the phosphorylation level of eIF2 α , preferably of a target host cell protein selected from EIF2AK2, EIF2AK3, DDX58, IFIH1, MAVS, IFNAR1, IFNAR2, IRF3, IRF7, IFNB1, TBK1,

20 TRAF2, TRAF3, IFIT1, JAK1, TYK2, STAT1, STAT2, IRF9, or protein phosphatase 1 PP1 or a subunit thereof, in particular PPP1CA or PPP1R15.
- 25 3. The method according to claim 2, wherein said polypeptide modulating the activity or the expression of a target host cell protein involved in the regulation of the phosphorylation level of eIF2 α is selected from:
 - (a) a viral protein selected from E3L of vaccinia virus, NSs from Rift Valley fever virus, N^{PRO} from Bovine Viral Diarrhea Virus, V protein from parainfluenza virus type 5, ICP34.5 from human Herpes-simplex virus-1, NS1 from Influenza A virus, NS1 protein from human respiratory syncytial virus, K3L of vaccinia virus, DP71L from African swine fever virus, in

30 particular the DP71(s) and DP71L(l) isoforms, VP35 from Zaire Ebolavirus, VP40 from the Marburg virus, LMP-1 from Epstein-Barr virus, μ 2 from reovirus, B18R of vaccinia virus, and ORF4a from Middle East respiratory syndrome coronavirus, a protein with at least 40% amino acid sequence identity with one of E3L of vaccinia virus, NSs from Rift Valley fever virus, N^{PRO} from Bovine Viral Diarrhea Virus, V protein from parainfluenza virus type 5,

35 ICP34.5 from human Herpes-simplex virus-1, NS1 from Influenza A virus, NS1 protein from human respiratory syncytial virus, K3L of vaccinia virus, DP71L from African swine fever virus, in particular the DP71(s) and DP71L(l) isoforms, VP35 from Zaire Ebolavirus, VP40 from the Marburg virus from the Marburg virus, LMP-1 from Epstein-Barr virus, μ 2 from

- reovirus, B18R of vaccinia virus and ORF4a from Middle East respiratory syndrome coronavirus, or a biologically active fragment thereof;
- (b) PPP1CA catalytic subunit and its regulatory proteins, in particular its host-cell regulatory proteins such as the eukaryotic protein PPP1R15, or a protein with at least 40% amino acid sequence identity with PPP1CA or PPP1R15, or a biologically active fragment thereof;
- (c) an inactive mutant of a host cell protein involved in the regulation of the phosphorylation level of eIF2 α , in particular selected from EIF2AK2 or EIF2AK3, or a biologically active fragment thereof, in particular the K296R mutant of the human EIF2AK2, or the dsRNA binding domain from EIF2AK2 deleted of its carboxy-terminal kinase domain, or a biologically active fragment thereof.
4. The method according to claim 2 or 3, wherein said polypeptide modulating the activity or the expression of a target host cell protein involved in the regulation of the phosphorylation level of eIF2 α is an eIF2AK2 inhibitor comprising at least one Z α domain, in particular a Z α domain from E3L of vaccinia virus or mammalian ADAR1 operably linked to at least one dsRNA-binding domain, in particular a dsRNA-binding domain from Influenza A virus NS1 protein, mammalian EIF2AK2, Flock House virus B2 protein, orthoreovirus σ 3 protein, preferably selected from Influenza A virus NS1 and mammalian EIF2AK2 proteins.
5. The method according to claim 2 or 3, wherein said polypeptide modulating the activity or the expression of a target host cell protein involved in the regulation of the phosphorylation level of eIF2 α is an eIF2AK2 inhibitor comprising:
- (a) the amino acid sequence set forth in SEQ ID NO. 16; or
 - (b) an amino acid sequence with at least 40% amino acid sequence identity with SEQ ID NO. 16; or
 - (c) a biologically active fragment of (a) or (b).
6. The method according to claim 2 or 3, wherein said polypeptide modulating the activity or the expression of a target host cell protein involved in the regulation of the phosphorylation level of eIF2 α is a chimeric protein comprising:
- a. a polypeptide capable of selectively binding to EIF2AK2, preferably selected from
 - dsRNA-binding region from EIF2AK2 protein deleted of its carboxyl-terminal kinase domain; or
 - orthologous dsRNA binding domains such as the dsRNA-binding domain of E3L protein from vaccinia virus; or
 - single-chain antibodies, such as nanobodies or ScFv, raised against EIF2AK2; and
 - b. a specific domain from multimeric E3 ligases, preferably selected from:

- Skp1-interacting domains from BTRCP, FBW7, SPK2; or
 - Elongin BC-interacting domains from VHL; or
 - Cullin3-interacting domains from SPOP; or
 - DDB1-interacting domains from CRBN or DDB2; or
 - Elongin BC-interacting domains from SOCS2; or
 - U-box interacting domain and coiled-coil dimerization domain from STUB1; or
 - CUL1-interacting domain from Skp1.
7. The method according to claim 1 or 2, wherein step (b) comprises introducing, into said host cell, at least two polypeptides, or one or more nucleic acid molecules encoding said polypeptides, wherein said polypeptides modulate the activity or the expression of at least two different target host cell proteins involved in the regulation of the phosphorylation level of eIF2 α , preferably wherein the modulation by said polypeptides has a supra-additive effect on the expression of said recombinant DNA by said host cell.
8. The method according to claim 7, wherein one of said at least two polypeptides inhibits the phosphorylation of eIF2 α , preferably is an EIF2AK2 inhibitor such as the dsRNA binding domain from EIF2AK2 deleted of its carboxy-terminal kinase domain, or a biologically active fragment thereof, and wherein another of said at least two polypeptides activates the dephosphorylation of eIF2 α , preferably is selected from PPP1CA or its viral and host-cell regulatory proteins, in particular PPP1R15, DP71L from African swine fever virus, such as its isoforms DP71L(s) or DP71L(l) and ICP34.5 from human Herpes-simplex virus-1 or a biologically active fragment thereof.
9. The method according to claim 1, wherein step (b) comprises introducing, into said host cell, a polypeptide comprising, the sequence of SEQ ID NO. 20 or SEQ ID NO. 36 or a sequence with at least 40% identity to SEQ ID NO. 20 or SEQ ID NO. 36, or a nucleic acid sequence encoding said polypeptide, wherein said polypeptide is capable of downregulating the phosphorylation level of eIF2 α .
10. The method according to any one of claims 1 to 9, wherein step (a) further comprises expressing at least one catalytic domain of a poly(A) polymerase, potentially tethered through a lambdoid N-peptide, in said host cell.
11. A eukaryotic host cell for the expression of a recombinant protein, characterized in that the phosphorylation level of eIF2 α is constitutively or transiently downregulated in said cell, and wherein said cell comprises at least one nucleic acid molecule encoding at least one chimeric protein comprising:

- (i) at least one catalytic domain of a capping enzyme, in particular selected in the group consisting of cap-0 canonical capping enzymes, cap-0 non-canonical capping enzymes, cap-1 capping enzymes and cap-2 capping enzymes; and
- (ii) at least one catalytic domain of a DNA-dependent RNA polymerase, in particular a bacteriophage DNA-dependent RNA polymerase.

12. A eukaryotic host cell according to claim 11, comprising a heterologous nucleic acid sequence encoding at least one polypeptide modulating the activity or the expression of a target host cell protein involved in the regulation of the phosphorylation level of eIF2 α , wherein said polypeptide is as defined in any one of claims 2 to 9.

13. The eukaryotic host cell according to claim 12, comprising a nucleic acid molecule comprising:
- at least one nucleic acid sequence encoding a chimeric protein comprising:
 - (i) at least one catalytic domain of a capping enzyme; and
 - (ii) at least one catalytic domain of a DNA-dependent RNA polymerase;
 - at least one nucleic acid sequence encoding at least one polypeptide modulating the activity or the expression of a target host cell protein involved in the regulation of the phosphorylation level of eIF2 α , wherein said polypeptide is as defined in any one of claims 2 to 9; and
 - optionally, at least one nucleic acid sequence encoding a poly(A) polymerase, potentially tethered through a lambdoid N-peptide.

14. An isolated nucleic acid molecule or a set of nucleic acid molecules, comprising or consisting of:
- (a) at least one nucleic acid sequence encoding a chimeric protein comprising:
 - (i) at least one catalytic domain of a capping enzyme, in particular selected in the group consisting of cap-0 canonical capping enzymes, cap-0 non-canonical capping enzymes, cap-1 capping enzymes and cap-2 capping enzymes; and
 - (ii) at least one catalytic domain of a DNA-dependent RNA polymerase; and
 - (b) at least one nucleic acid sequence downregulating the phosphorylation level of eIF2 α in a eukaryotic host cell, or encoding a polypeptide downregulating said phosphorylation level; and,
 - (c) optionally, at least one nucleic acid sequence encoding a poly(A) polymerase, potentially tethered through a lambdoid N-peptide.

15. The isolated nucleic acid molecule or set of nucleic acid molecules according to claim 14, wherein said nucleic acid sequence downregulating the phosphorylation level of eIF2 α encodes at least one polypeptide modulating the activity or the expression of a target host cell protein involved in the regulation of the phosphorylation level of eIF2 α in a eukaryotic host cell,

preferably modulating the activity or the expression of a target host cell protein selected from EIF2AK2, EIF2AK3, DDX58, IFIH1, MAVS, IFNAR1, IFNAR2, IRF3, IRF7, IFNB1, TBK1, TRAF2, TRAF3, IFIT1, a type-I interferon protein, JAK1, TYK2, STAT1, STAT2, IRF9, or protein phosphatase 1 PP1 or a subunit thereof, in particular PPP1CA or PPP1R15.

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16. The isolated nucleic acid molecule or set of nucleic acid molecules according to claim 14 or 15, wherein said polypeptide modulating the activity or the expression of a target host cell protein involved in the regulation of the phosphorylation level of eIF2 α is selected from:

- 10 (a) a viral protein selected from E3L from vaccinia virus, NSs from Rift Valley fever virus, N^{PRO} from Bovine Viral Diarrhea Virus, V protein from parainfluenza virus type 5, ICP34.5 from human Herpes-simplex virus-1, NS1 from influenza A virus, K3L from vaccinia virus, DP71L from African swine fever virus, in particular the DP71(s) and DP71L(l) isoforms, VP35 from Zaire Ebolavirus, VP40 from Marburg virus, LMP-1 from Epstein-Barr virus, μ 2 from reovirus, B18R from vaccinia virus and ORF4a from Middle East respiratory syndrome coronavirus, a protein with at least 40% amino acid sequence identity with one of E3L from vaccinia virus, NSs from Rift Valley fever virus, N^{PRO} from Bovine Viral Diarrhea Virus, V protein from parainfluenza virus type 5, ICP34.5 from human Herpes-simplex virus-1, NS1 from influenza A virus, K3L from vaccinia virus, DP71L from African swine fever virus, in particular the DP71(s) and DP71L(l) isoforms, VP35 from Zaire Ebolavirus, VP40 from Marburg virus, LMP-1 from Epstein-Barr virus, μ 2 from reovirus, B18R from vaccinia virus and ORF4a from Middle East respiratory syndrome coronavirus, or a biologically active fragment thereof;
- 15 (b) PPP1CA catalytic subunit and its regulatory proteins, in particular host-cell regulatory proteins such as the eukaryotic protein PPP1R15, or a protein with at least 40% amino acid sequence identity with PPP1CA or PPP1R15, or a biologically active fragment thereof;
- 20 (c) an inactive mutant of a host cell protein involved in the regulation of the phosphorylation level of eIF2 α , in particular selected from EIF2AK2 or EIF2AK3 or a biologically active fragment thereof, in particular the K296R mutant of the human EIF2AK2 or a biologically active fragment thereof.

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17. The isolated nucleic acid molecule or set of nucleic acid molecules according to claim 15 or 16, wherein said polypeptide modulating the activity or the expression of a target host cell protein involved in the regulation of the phosphorylation level of eIF2 α is an eIF2AK2 inhibitor comprising at least one Z α domain, in particular a Z α domain from E3L of vaccinia virus or mammalian ADAR1 operably linked to at least one dsRNA-binding domain, in particular a dsRNA-binding domain from Influenza A virus NS1 protein, mammalian EIF2AK2, Flock House virus B2 protein, orthoreovirus σ 3 protein, preferably selected from Influenza A virus NS1 and mammalian EIF2AK2 proteins.

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18. The isolated nucleic acid molecule or set of nucleic acid molecules according to any one of claims 14 to 17, comprising, from the 5'-terminus to the 3'-terminus:
- said at least one nucleic acid sequence encoding a catalytic domain of a poly(A) polymerase potentially tethered through a lambdoid N-peptide;
 - 5 – said at least one nucleic acid sequence encoding said polypeptide downregulating the phosphorylation level of eIF2 α ; and
 - said at least one nucleic acid sequence encoding a chimeric protein comprising:
 - (i) at least one catalytic domain of a capping enzyme; and
 - (ii) at least one catalytic domain of a DNA-dependent RNA polymerase.
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19. The isolated nucleic acid molecule or set of nucleic acid molecules according to any one of claims 14 to 17, comprising, from the 5'-terminus to the 3'-terminus:
- said at least one nucleic acid sequence encoding said polypeptide downregulating the phosphorylation level of eIF2 α ;
 - 15 – said at least one nucleic acid sequence encoding a catalytic domain of a poly(A) polymerase potentially tethered through a lambdoid N-peptide; and
 - said at least one nucleic acid sequence encoding a chimeric protein comprising:
 - (iii) at least one catalytic domain of a capping enzyme; and
 - (iv) at least one catalytic domain of a DNA-dependent RNA polymerase.
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20. A kit for the production of a recombinant protein of interest from a recombinant DNA, comprising or consisting of:
- (a) an isolated nucleic acid molecule or a set of isolated nucleic acid molecules comprising:
 - (i) a nucleic sequence encoding a chimeric protein comprising:
 - 25 ○ at least one catalytic domain of a capping enzyme; and
 - at least one catalytic domain of a DNA-dependent RNA polymerase; and
 - (ii) optionally, at least one nucleic acid sequence encoding a poly(A) polymerase potentially tethered through a lambdoid N-peptide; and
 - (b) at least one compound capable of downregulating the phosphorylation level of eIF2 α in a
- 30 eukaryotic host cell.
21. A polyprotein, polypeptide or a set of polypeptides encoded by the isolated nucleic acid molecule or set of nucleic acid molecules of any one of claims 14 to 19.
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22. Use of an isolated nucleic acid molecule or a set of isolated nucleic acid molecules according to any one of claims 14 to 19, a kit according to claim 20, a polyprotein, polypeptide, or set of polypeptides according to claim 21 and/or a cell according to any one of claims 11 to 13, for the *in vitro* or *ex vivo* or *in cellulo* production of a recombinant protein.

23. A pharmaceutical composition comprising an isolated nucleic acid molecule or a set of isolated nucleic acid molecules according to any one of claims 14 to 19, a kit according to claim 20, a polyprotein, polypeptide, or set of polypeptides according to claim 21 and/or a cell according to any one of claims 11 to 13.
24. An isolated nucleic acid molecule or a set of isolated nucleic acid molecules according to any one of claims 14 to 19, a kit according to claim 20, a polyprotein, polypeptide, or set of polypeptides according to claim 21, a cell according to any one of claims 11 to 13, and/or a pharmaceutical composition according to claim 23, for use in combination with a nucleic acid encoding an antigen or a therapeutic agent, in a method of treatment or prophylaxis, in particular for gene therapy or for generating an immune response against an antigen in an individual.