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(54) Title: POSITIVE CYTOMODULINES TO IMPROVE BIOREACTOR PRODUCTIVITY.

(57) Abstract: The present invention relates to the activation of the protein synthesis by controlling the post-transcriptional steps of mRNA translation into proteins. A polypeptide is provided, which is capable of activating said protein translation. The principal industrial application of the invention is the control of recombinant protein production in cultured cells including in bioreactors.

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Positive cytomodulines to improve bioreactor productivity

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FIELD OF THE INVENTION

The present invention relates to the activation of the protein synthesis by controlling the post-transcriptional steps of the mRNA translation into proteins. The principal industrial application of the invention is the control of the protein production in bioreactors.

10

BACKGROUND OF THE INVENTION

Improvements in the production of recombinant proteins using eukaryotic cells are of major importance, in particular for the use of these cells in bioreactors. The production of proteins in bioreactors is expected to increase about 4 fold between 2003 and 2010 ("Optimisation de l'attractivité de la France pour la production biologique" realised in 2004 by the Arthur Doolittle Office for the pharmaceutical companies - LEEM).

20

The naturally occurring *in vivo* production of proteins in cellular systems is now well known for the skilled person : the pre-messenger RNAs (pre-mRNAs) are transcribed in the nucleus from genomic sequences. They are then matured into mRNAs by the processes of splicing, 3' polyadenylation and 5' capping and subsequently exported to the cytoplasm where they are used by the translation machinery to direct protein synthesis.

25

The cap added to the 5' end of the mRNA is a methylated guanosine triphosphate. In the cytoplasm this modified nucleotide is specifically recognised by the protein complex eIF4F that is required for the recruitment of the small 40S ribosomal subunit to the 5'

extremity of the mRNA. Once recruited, the 40S ribosomal subunit moves along the mRNA until a methionine codon (AUG) is encountered. Translation is initiated at this codon after the association of the second ribosomal subunit (60S). Hence, any molecule that increases the efficiency of this chain of events will increase translational efficiency.

5 For a small number of cellular viral mRNAs, the translation initiation uses a mechanism that short cuts the 5' Cap. The conception of the present invention does not use this mode of translational initiation and accordingly it will not be presented here.

The complex eIF4F contains several translation initiation factors including the proteins eIF4E, that binds to the 5' Cap, and eIF4G (Wickens et al., 2000). This latter factor acts
10 as a platform that allows the assembly of the eIF4F complex. The protein eIF4G can associate with several other proteins, eIF4E as already mentioned, and the poly(A) binding protein (PABP) that binds to the polyadenosine sequence added to the 3' extremity of the mRNA (see Figure 1). The association of the PABP with eIF4G leads to a circularisation of the mRNA, a stabilisation of the eIF4F complex and simulation of
15 cap-dependent translation (Wickens et al., 2000). Any event that destabilises the eIF4F complex causes a decrease in translation. Hence, the loss of the 3' poly(A) tail by an mRNA causes a loss of the PABPs bound to that mRNA which is associated with a decreased translation of the mRNA (Kahvejian et al., 2001; Kahvejian et al., 2005). In contrast, the translation of an mRNA is stimulated when the length of a poly(A) tail
20 increases, an event that causes an increases the number of PABPs associated with the mRNA (Richter, 1999). In this case it would appear that the increased length of the poly(A) tail leads to a greater number of associated PABPs that are available to interact with eIF4G which in turn stimulates translation. It should be noted that the association of the PABP with the poly(A) tail, in addition to the effect on translation described above,
25 also stabilises the mRNA. In cells depleted of PABP the translation is decreased and the degradation of the mRNAs is increased.

Although many aspects of the naturally occurring *in vivo* production of proteins are well known, the step to apply this knowledge in industrial applications, such as to boost the production of recombinant proteins on a large scale, has not been taken in a generalised manner.

5

SUMMARY OF THE INVENTION

The inventors have surprisingly discovered that the use of the Non-Structural Protein 3, which is present in rotaviruses, could respond to the industrial requirements of eukaryotic recombinant protein production.

Rotaviruses (Reoviridae family) infect mammalian cells and use the endogenous translation machinery to synthesize the viral proteins (Padilla-Noriega et al., 2002). The viral mRNAs have a 5' cap but no 3' poly(A) tail. Instead, they code for the protein NSP3 (Non-structural protein 3) which, like PABP, binds to a specific sequence at the 3' end of the viral mRNA and to the protein eIF4G (Piron et al., 1998). The interaction between NSP3 and eIF4G occurs via the same domain of eIF4G that interacts with PABP (Piron et al., 1998). In addition, as for the eIF4G-PABP interaction, the complex NSP3-eIF4G stabilises the interaction of eIF4G with eIF4E and consequently the interaction of eIF4E with the cap (Vende et al., 2000). Hence, the protein NSP3 can functionally replace PABP in its role as a translational stimulator. The interaction of NSP3 with RNA and eIF4G occurs via two separate domains (Piron et al., 1999). The crystal structures of each of these domains, associated with the appropriate ligands, have been published (Deo et al., 2002; Groft and Burley, 2002).

The inventors have developed the cytomoduline concept (positive cytomoduline, or cytoboost project) that are hybrid protein molecules, also named herein chimeric

polypeptides or proteins, composed in two parts that are functionally distinct, a domain that can specifically interact with an RNA and an effector domain. The RNA-binding domain associates with a mRNA containing the target sequence. This domain acts as a tether which specifically attaches the molecule to the target mRNA. The second domain
5 is the effector domain, which for the cytoboost project, causes a stimulation of the translation of the target mRNA. By varying either the tethering (RNA-binding) or the effector domain it is possible to change, respectively, the RNA target of the cytomoduline or the effect obtained.

As indicated, Cytomodulines are hybrid molecules composed of two domains that are
10 functionally distinct: a tethering and an effector domain (see Figure 2).

The tethering domain of a Cytomoduline binds to an mRNA with sequence specificity. This is necessary for the Cytomoduline to express its function as a regulator of the translation or the stability of targeted mRNAs. Tethering domains can be obtained in several ways. In the present case where cytomodulines are used to increase the
15 production in bioreactors, the Cytomoduline must recognise a recombinant mRNA which gives a greater liberty in the choice of the tethering domain and target sequence. A certain number of RNA-binding proteins have been described in diverse organisms such as eukaryotic cells, virus, bacteriophages and bacteria. Using a tethering domain from a protein normally expressed in eukaryotic cells leads to a problem of Cytomoduline
20 specificity. These molecules would not only bind to the recombinant target mRNA but also to cellular mRNAs that are the endogenous targets of the protein. RNA-binding proteins encoded by the genome of bacteriophages have been chosen.

The effector domain of a positive Cytomoduline must interact with the translation machinery in such a way that the translation is stimulated. To achieve this interaction it
25 should either stabilise or assist in the formation of the ribonucleo-protein complexes of the translation machinery. The present status of research on translational control indicates that regulations exist mainly at the level of the recognition of the 5' Cap or the recruitment of the 40S ribosomal subunit.

The positive Cytomoduline of the invention is based on an effector domain derived from the protein NSP3 that is composed of three domains:

- a N-terminal domain that binds to RNA as a dimer.
- a central domain that appears to be important for the dimerisation.
- 5 - a C-terminal domain that binds to eIF4G.

The inventors have thus obtained “positive cytomodulines” (“cytoboost project”) or “positive chimeric polypeptides” which are able to activate protein translation. Active molecules (positive cytomodulines) that specifically increase the expression of target proteins in eukaryotic cells are the subject of the present invention. These molecules are effective in bioreactor conditions, for the protein production maximised by the appropriate structural modifications.

An industrial/economic development is proposed in the form of a kit that contains an expression plasmid to receive the gene of interest and a cell line that stably expresses the positive cytomoduline for the application.

15

The NSP3 protein to be used in the invention may be selected from the NSP3 of the Avian rotavirus A, preferably avian rotavirus AvRV-1, avian rotavirus Ch-1, avian rotavirus PO-13, avian rotavirus RK3, avian rotavirus Ty-1, avian rotavirus Ty-2, or avian rotavirus Ty-3. It may be also a NSP3 protein of the Bovine rotavirus A, such as bovine rotavirus 993/83 or bovine rotavirus RF, caprine rotavirus A, equine rotavirus A, human rotavirus A, preferably human rotavirus (serotype P13 / strain 1845), human rotavirus 1, human rotavirus 2, human rotavirus 4, human rotavirus DG8, human rotavirus G1, G10, G12, G2, G3, G4, G6, G8, or G9, human rotavirus P1B, human rotavirus P3, human rotavirus RMC321, or else untyped human rotaviruses. The NSP3 protein may be further selected from the NSP3 of the lapine rotavirus, such as lapine rotavirus strain BAP (wildtype), lapine rotavirus strain BAP-2, lapine rotavirus strain C-11, lapine rotavirus strain R-2, or rotavirus str. ALA, but also to porcine rotavirus A, preferably porcine rotavirus A strain 134/04-15, rabbit rotavirus, preferably rabbit

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rotavirus (STRAIN ALABAMA), and rotavirus 5 serotype G2, rotavirus G8, rotavirus subgroup 1, rotavirus subgroup 2, simian rotavirus A/SA11, simian 11 rotavirus (serotype 3 / strain SA11-Patton), simian 11 rotavirus (serotype 3 / strain SA11-Ramig), simian rotavirus, simian rotavirus A/SA11-4F, simian rotavirus A/SA11-both, simian
5 rotavirus A/SA11-C14, simian rotavirus A/SA11-FEM, simian rotavirus A/SA11-SEM, swine rotavirus strain S8, or else unclassified Rotavirus A, such as canine rotavirus, canine rotavirus serotype P13 / strain K9, canine rotavirus strain CU-1, lamb rotavirus, rhesus rotavirus, rotavirus GB-503, rotavirus GB-5737, rotavirus RattG1, rotavirus str. I321, rotavirus strain TUCH, and rotavirus TK159.

10 Thus, the NSP3 protein may be derived from all the group A rotaviruses of the above non-limiting list.

In particular, the NSP3 which is used in the present invention is the NSP3 protein of the group A Bovine rotavirus strain RF whose amino-acid sequence is accessible online at the
15 address <http://www.ncbi.nlm.nih.gov/> under the numbers CAA79754 and Z21639 (nucleotide sequence). These amino-acid and nucleotide sequences are disclosed herein as the sequences SEQ ID N°46 and SEQ ID N°47, respectively.

Another preferred NSP3 which may be used in the present invention is that of the group
20 A rotavirus strain Simian SA11, preferably the Simian rotavirus A/SA11-C14, whose amino-acid sequence is accessible online at the address <http://www.ncbi.nlm.nih.gov/> under the numbers AAL58537 and AY065843 (nucleotide sequence). These amino-acid and nucleotide sequences are disclosed herein as the sequences SEQ ID N°29 and SEQ ID N°30, respectively.

25

Moreover, the inventors have determined minimal regions required for an efficient stimulation of the translation positive cytomoduline of the invention (“chimeric

polypeptide”), in order to avoid that the NSP3 protein also binds to other proteins. The maximal specificity of the positive cytomoduline has been thus determined.

They have shown that some particular fragments of the NSP3 protein of the group A
5 Bovine rotavirus strain RF have the capacity to activate/stimulate protein translation. These peptides are the following: amino acids 10 – 313; 150 – 313; 206 – 313; 229 – 313; 250 – 313; 272 – 313; 283 – 313, and amino acids 6 – 313; 146 – 313; 202 – 313; 225 – 313; 246 – 313; 268 – 313; 279 – 313. The schematic organisation of all these preferred peptides is given in the Figure 6 and the amino-acid sequences thereof is given
10 in the sequence listing, from the sequence SEQ ID N°32 to the sequence SEQ ID N°45. Sequences having at least 70 %, preferably 75 %, 80 %, 85 %, 90 %, 95 %, 96 %, 97 %, 98 % or 99 % of identity with said sequences SEQ ID N°32 to 45 are also within the scope of the invention.

15 Another fragment of the NSP3 protein of the group A Bovine rotavirus strain RF corresponds to the amino-acids 163 to 313 of the sequence SEQ ID N°46.

Similarly, some particular fragments of the NSP3 protein of the group A Simian rotavirus strain A/SA11-C14 have the capacity to activate/stimulate protein translation. These
20 peptides are the following: amino acids 10 – 315; 150 – 315; 206 – 315; 229 – 315; 250 – 315; 272 – 315; 283 – 315, amino acids 10 – 313; 150 – 313; 206 – 313; 229 – 313; 250 – 313; 272 – 313; 283 – 313, amino acids 6 – 315; 146 – 315; 202 – 315; 225 – 315; 246 – 315; 268 – 315; 279 – 315, and amino acids 6 – 313; 146 – 313; 202 – 313; 225 – 313; 246 – 313; 268 – 313; 279 – 313. The schematic organisation of these peptides
25 derived from the Simian rotavirus A/SA11-C14 strain is given in the Figure 5, and the amino-acid sequences thereof is given in the sequence listing, from the sequence SEQ ID N°1 to the sequence SEQ ID N°28. Sequences having at least 70 %, preferably 75 %, 80

%, 85 %, 90 %, 95 %, 96 %, 97 %, 98 % or 99 % of identity with said sequences SEQ ID N°1 to 28 are also within the scope of the invention.

Table 3 below indicates identity scores for some rotavirus group A NSP3 proteins.

5

The minimal region required to increase protein production will be used to make modification aimed at modulating the effect of the cytomoduline. An increased efficiency is important for their use in bioreactors. Site directed mutagenesis of the amino acids that participate either in the interface between NSP3 and eIF4G or in the structuring of that interface should affect the mutual affinity of these two proteins and hence the degree to which translation is stimulated.

10

15 **DETAILED DESCRIPTION**

In a first aspect, the invention is aimed at a polypeptide comprising or consisting of the amino-acid sequence SEQ ID N°32 or a sequence having at least 70 % of identity with SEQ ID N°32, or any one of its fragments, which is capable of activating protein translation.

20

The terms protein, polypeptide and peptide are used indifferently herein to design an amino acid sequence or their derivatives or analogues containing an amino acid sequence. In the same way, the expressions nucleic acid, nucleotidic acid, polynucleotide, etc.. are used indifferently herein to design a nucleic acid sequence.

25

By percentage of identity between two amino acid or nucleic acid sequences in the present invention, it is meant a percentage of identical amino acid residues or nucleotides

between the two sequences to compare, obtained after the best alignment ; this percentage is purely statistical, and the differences between the two sequences are randomly distributed and all along their length. The best alignment or optimal alignment is the alignment corresponding to the highest percentage of identity between the two sequences to compare, which is calculated such as herein after. The sequence
5 comparisons between two nucleic acid or amino acid sequences are usually performed by comparing these sequences after their optimal alignment, said comparison being performed for one segment or for one “comparison window”, to identify and compare local regions of sequence similarity. The optimal alignment of sequences for the comparison can be performed manually or by means of the algorithm of local homology
10 of Smith and Waterman (1981) (Ad. App. Math. 2:482), by means of the algorithm of local homology of Neddleman and Wunsch (1970) (J. Mol. Biol. 48:443), by means of the similarity research method of Pearson and Lipman (1988) (Proc. Natl. Acad. Sci. USA 85:2444), by means of computer softwares using these algorithms (GAP,
15 BESTFIT, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI).

The percentage of identity between two amino acid or nucleic acid sequences is determined by comparing these two aligned sequences in an optimal manner with a “comparison window” in which the region of the nucleic acid or amino acid sequence to
20 compare may comprise additions or deletions with regard to the sequence of reference for an optimal alignment between these two sequences. The percentage of identity is calculated by determining the number of positions for which the nucleotide or the amino acid residue is identical between the two sequences, by dividing this number of identical positions by the total number of positions in the “comparison window” and by
25 multiplying the result obtained by 100, to obtain the percentage of identity between these two sequences.

In a preferred embodiment, the fragment of the amino-acid sequence SEQ ID N°32 or a sequence having at least 70 % of identity with SEQ ID N°32, is selected from the group consisting of the fragments of sequence SEQ ID N°33 and sequences having at least 70 % of identity with SEQ ID N°33.

5

In another preferred embodiment, the fragment of the amino-acid sequence SEQ ID N°32 or a sequence having at least 70 % of identity with SEQ ID N°32, is selected from the group consisting of the fragments of sequence SEQ ID N°34, SEQ ID N°35 and sequences having at least 70 % of identity with SEQ ID N°34 and SEQ ID N°35.

10

In another preferred embodiment, the fragment of the amino-acid sequence SEQ ID N°32 or a sequence having at least 70 % of identity with SEQ ID N°32, is selected from the group consisting of the fragments of sequence SEQ ID N°36, SEQ ID N°37 and sequences having at least 70 % of identity with SEQ ID N°36 and SEQ ID N°37.

15

In another preferred embodiment, the fragment of the amino-acid sequence SEQ ID N°32 or a sequence having at least 70 % of identity with SEQ ID N°32, is selected from the group consisting of the fragments of sequence SEQ ID N°38, SEQ ID N°39 and sequences having at least 70 % of identity with SEQ ID N°38 and SEQ ID N°39.

20

In another preferred embodiment, the fragment of the amino-acid sequence SEQ ID N°32 or a sequence having at least 70 % of identity with SEQ ID N°32, is selected from the group consisting of the fragments of sequence SEQ ID N°40, SEQ ID N°41 and sequences having at least 70 % of identity with SEQ ID N°40 and SEQ ID N°41.

25

In another preferred embodiment, the fragment of the amino-acid sequence SEQ ID N°32 or a sequence having at least 70 % of identity with SEQ ID N°32, is selected from

the group consisting of the fragments of sequence SEQ ID N°42, SEQ ID N°43 and sequences having at least 70 % of identity with SEQ ID N°42 and SEQ ID N°43.

In another preferred embodiment, the fragment of the amino-acid sequence SEQ ID N°32
5 or a sequence having at least 70 % of identity with SEQ ID N°32, is selected from the group consisting of the fragments of sequence SEQ ID N°44, SEQ ID N°45 and sequences having at least 70 % of identity with SEQ ID N°44 and SEQ ID N°45.

Preferably, the sequences having at least 70 % of identity with anyone of sequences SEQ
10 ID N°32 to SEQ ID N°45 originate from anyone of the following rotaviruses : bovine rotavirus A, caprine rotavirus A, equine rotavirus A, human rotavirus A, lapine rotavirus, porcine rotavirus A, rabbit rotavirus, rotavirus 5 serotype G2, rotavirus G8, rotavirus subgroup 1, rotavirus subgroup 2, simian rotavirus A/SA11, simian rotavirus A/SA11-C14 and swine rotavirus strain S8. Preferably, it originates from the simian rotavirus
15 A/SA11-C14 (accession number: AAL58537) and the corresponding preferred fragments are disclosed above and in the Figure 5.

The activating polypeptides as defined above are used to activate in a non specific manner protein translation, preferably in cell systems, although the activating polypeptides may
20 also be used to activate protein translation in acellular systems, such as for example by incubating a cellular mRNA extract constituted of a rabbit reticulocyte lysate according to the conventional method described in UCHIDA et al (2002)

In a second aspect, the invention relates to a chimeric polypeptide capable of specifically
25 activating the translation of a target polynucleotide of interest, which comprises or consists of the Non-Structural Protein 3 (NSP3) of sequence SEQ ID N°46 or a sequence having at least 70 %, preferably 75 %, 80 %, 85 %, 90 %, 95 %, 96 %, 97 %, 98 % or 99

% of identity with SEQ ID N°46 or anyone of the fragments thereof, wherein said NSP3 or anyone of its fragments is fused to a RNA binding protein.

The chimeric polypeptide as defined above is used to activate in a specific manner the translation of a target polynucleotide of interest in the corresponding protein.

Preferably, the fragment of the NSP3 protein is the polypeptide according to the present invention, said polypeptide comprising or consisting of the amino-acid sequence SEQ ID N°32 or a sequence having at least 70 % of identity with SEQ ID N°32, or anyone of its fragments. Preferably, the fragment is selected from the group consisting of the fragments of sequence SEQ ID N°33 to SEQ ID N°45, and sequences having at least 70 % of identity with the sequences SEQ ID N°33 to SEQ ID N°45.

Another preferred chimeric polypeptide of the present invention comprises or consists of the sequence SEQ ID N°48 or a sequence having at least 70% of identity with SED ID N°48.

The RNA binding protein of the chimeric polypeptide according to the present invention may be one of the proteins as disclosed in the Table 1 below:

Table 1:

RNA binding protein	Reference
MS2	J. COLLER et al., Genes Dev. 1998 Oct 15;12(20):3226-35; WITHERELL et al. Prog Nucleic Acid Res Mol Biol. 1991;40:185-220
N	DE GREGORIO et al., EMBO J. 1999 Sep 1;18(17):4865-74; TAN & FRANKEL, Biochemistry. 1994 Dec 6;33(48):14579-85
IRP	DE GREGORIO et al., RNA. 2001 Jan;7(1):106-13 ; HENZE & KUHN, Proc Natl Acad Sci U S A. 1996 Aug 6;93(16):8175-82. Review
U1A	BRODSKY & SILVER, RNA. 2000 Dec;6(12):1737-49

Thus, in one further embodiment, the invention is directed to a chimeric polypeptide according to the present invention, wherein the RNA binding protein is selected from the group consisting of MS2CP, N, IRP and U1A. Preferably, the RNA binding protein is MS2CP containing mutations or deletions that retain the ability to dimerise but which prevent aggregation (Peabody and Ely, 1992; Le Cuyer et al., 1995) or genetic fusions of two MS2CP coding sequences (Peabody and Lim, 1996) containing or not the
5 aforementioned mutations or deletions.

Other RNA binding proteins not listed in Table 1 are also within the scope of the
10 invention.

Advantageously, the selected RNA binding protein is localised at the N-terminal extremity of the chimeric polypeptide, although it may be also localised at the C-terminal extremity of said chimeric polypeptide.

15 In the chimeric polypeptide, the RNA binding protein may be fused directly to the effector domain, which means:

(i) when the RNA binding protein is localised at the N-terminal part of the chimeric polypeptide, the last amino-acid localised in C-terminal position of the RNA binding protein is chemically linked, preferably by a normal peptidic linkage, to the amino-acid
20 localised at the N-ter position of the effector domain; or

(ii) when the RNA binding protein is localised at the C-terminal part of the chimeric polypeptide, the amino-acid localised in C-terminal position of the effector domain is chemically linked, preferably by a normal peptidic linkage, to the amino-acid localised at the N-ter position of the RNA binding protein.

25 In another embodiment, the RNA binding protein and the effector domain are not directly linked with each other, but are on the contrary separated in the chimeric polypeptide by a spacer amino-acid sequence or “linker”, which is preferably hydrophobic. The spacer

amino-acid sequence has a size which is sufficient to constitute a flexible region of the amino-acid molecule, allowing a relative mobility of the RNA binding protein.

The spacer sequence is between 3 and 50 amino-acids long, preferably between 5 and 30 amino-acids long, and most preferably between 5 and 20 amino-acids long.

5 Preferably, when the spacer sequence or spacer peptide is hydrophobic, said spacer peptide makes it easier for the polypeptide to penetrate the cell membrane. In such a case, said spacer peptide contains a majority of hydrophobic amino-acids such as the amino-acids valine, leucine or isoleucine.

In this embodiment, the spacer peptide comprises in its sequence preferably at least 50 %
10 of hydrophobic amino-acids, more preferably at least 60 % of hydrophobic amino-acids, most preferably at least 80 % of hydrophobic amino-acids.

In a particular embodiment, the spacer peptide consists in a poly(alanine) amino-acid chain, comprising from 3 to 50, preferably from 5 to 30, more preferably from 5 to 20 and most preferably from 5 to 10 alanine amino-acids.

15 In another embodiment, the spacer peptide consists of 3 to 50 amino acids and most preferably 5 to 10 amino-acids the sequence of which does not modify the function of the cytomoduline. For example, said spacer peptide may consist in the sequence SEQ ID N°50.

In still another embodiment, the spacer peptide is a tag allowing the detection or the
20 purification of the chimeric polypeptide present in a sample. For example, the spacer peptide may consist in the "HA TAG" peptide of sequence SEQ ID N°31.

In a more preferred embodiment, the sequences having at least 70 % of identity with anyone of sequences SEQ ID N°32 to SEQ ID N°46 originate from anyone of the
25 following rotaviruses : bovine rotavirus A, caprine rotavirus A, equine rotavirus A, human rotavirus A, lapine rotavirus, porcine rotavirus A, rabbit rotavirus, rotavirus 5 serotype G2, rotavirus G8, rotavirus subgroup 1, rotavirus subgroup 2, simian rotavirus A/SA11, simian rotavirus A/SA11-C14 and swine rotavirus strain S8. Preferably, it

originates from the simian rotavirus A/SA11-C14 (accession number: AAL58537) and the corresponding preferred fragments are disclosed above and in the Figure 5.

Preferably, the NSP3 protein to be used in the invention is selected from the NSP3 of the
5 Avian rotavirus A, preferably avian rotavirus AvRV-1, avian rotavirus Ch-1, avian rotavirus PO-13, avian rotavirus RK3, avian rotavirus Ty-1, avian rotavirus Ty-2, or avian rotavirus Ty-3. It may be also a NSP3 protein of the Bovine rotavirus A, such as bovine rotavirus 993/83, caprine rotavirus A, equine rotavirus A, human rotavirus A, preferably human rotavirus (serotype P13 / strain 1845), human rotavirus 1, human
10 rotavirus 2, human rotavirus 4, human rotavirus DG8, human rotavirus G1, G10, G12, G2, G3, G4, G6, G8, or G9, human rotavirus P1B, human rotavirus P3, human rotavirus RMC321, or else untyped human rotaviruses. The NSP3 protein may be further selected from the NSP3 of the lapine rotavirus, such as lapine rotavirus strain BAP (wildtype), lapine rotavirus strain BAP-2, lapine rotavirus strain C-11, lapine rotavirus strain R-2, or
15 rotavirus str. ALA, but also to porcine rotavirus A, preferably porcine rotavirus A strain 134/04-15, rabbit rotavirus, preferably rabbit rotavirus (STRAIN ALABAMA), and rotavirus 5 serotype G2, rotavirus G8, rotavirus subgroup 1, rotavirus subgroup 2, simian rotavirus A/SA11, simian 11 rotavirus (serotype 3 / strain SA11-Patton), simian 11 rotavirus (serotype 3 / strain SA11-Ramig), simian rotavirus, simian rotavirus A/SA11-
20 4F, simian rotavirus A/SA11-both, simian rotavirus A/SA11-C14, simian rotavirus A/SA11-FEM, simian rotavirus A/SA11-SEM, swine rotavirus strain S8, or else unclassified Rotavirus A, such as canine rotavirus, canine rotavirus serotype P13 / strain K9, canine rotavirus strain CU-1, lamb rotavirus, rhesus rotavirus, rotavirus GB-503, rotavirus GB-5737, rotavirus RattG1, rotavirus str. I321, rotavirus strain TUCH, and
25 rotavirus TK159.

Preferably, the chimeric polypeptide according to the present invention further comprises a transport signal which allows the chimeric polypeptide to cross the plasma membrane of

the target cells. Transport signals are well known from the skilled person. It may be for example spacer peptides as described above.

In a third aspect, the invention relates to a nucleic acid comprising or consisting of a polynucleotide encoding a polypeptide according to the present invention. Preferably, 5 said nucleic acid further comprises an inducible regulatory polynucleotide for the control of the polynucleotide encoding the polypeptide according to the present invention.

In a fourth aspect, the invention relates to a nucleic acid comprising or consisting of a polynucleotide encoding a chimeric polypeptide according to the present invention. In an 10 advantageous embodiment, said nucleic acid comprises or consists of the sequence SEQ ID N°49. Preferably, said nucleic acid further comprises an inducible regulatory polynucleotide for the control of the polynucleotide encoding the chimeric polypeptide according to the present invention.

15

The invention also relates to a nucleic acid comprising a polynucleotide encoding a chimeric polypeptide as defined herein and which also comprises a regulatory polynucleotide sensitive to the direct or indirect action of an inducer signal or agent, also disclosed herein as an inducible regulatory polynucleotide.

20 In practice, the control of the translation of certain predetermined target proteins by a positive chimeric polypeptide according to the invention implies that at particular moments of a cell culture, for example in a bioreactor, the target protein(s) is(are) not produced, whereas at another moments the production is on contrary searched for.

Preferred inducible regulation systems are listed in the Table 2 below:

25

Table 2:

NAME	PROMOTER	INDUCER	BIBLIOGRAPHICAL OR COMMERCIAL REFERENCES
pMSG	MMTV-LTR « (mouse mammary tumor virus »)	Dexamethasone	Amersham Pharmacia
pOPRSVI/MCS	RSV-LTR (“Rous sarcoma virus”)	IPTG	Stratagene
pTet-Splice	Tet	Tetracycline	Life Technologies
pTRE	hCMV-1	Tetracycline or doxycycline	Clontech
pRev-TRE	hCMV-1	Tetracycline or doxycycline	Clontech
² pRetro-On pRetro-Off	hCMV-1	Tetracycline or doxycycline	Clontech
pIND series	Δ HSP (« Heat shock protein »)	Ecdysone	Invitrogen
pPOP	mPGK/lacO (phosphoglycérate kinase)	IPTG	G.N. Hannan, S.A. Lehnert, E.S. MacAvoy, P.A. Jennings and P.L. Molloy, An engineered <i>PGK</i> promoter and <i>lac</i> operator-repressor system for the regulation of gene expression in mammalian cells. <i>Gene</i> 130 (1993), pp. 233-239.
pEF-LAC	hEF-1 α /lacO	IPTG	Edamatsu, H., Kaziro,

			Y., and Itoh, H. (1997) Inducible high level expression vector for mammalian cells, pEF-LAC carrying human elongation factor 1 α promoter and lac operator. <i>Gene</i> 187 , 289-294.
pBPVMT1	mMT-I (metallothionein I)	Cd ⁺⁺ , Zn ⁺⁺ , PMA ⁺⁺	Pavlakakis, G.N., and Hamer, D.H. (1983) Regulation of a metallothionein-growth hormone hybrid gene in bovine papilloma virus. <i>Proc. Natl. Acad. Sci. USA</i> 80 , 397-401
pMT	hMT-II (metallothionein II)	Cd ⁺⁺ , Zn ⁺⁺ , PMA ⁺⁺	Friedman, J.S., Cofer, C.L., Anderson, C.L., Kushner, J.A., Gray, P.P., Chapman, G.E., Stuart, M.C., Lazarus, L., Shine, J., and Kushner, P.J. (1989) High expression in mammalian cells without amplification. <i>Bio/Technology</i> 7 , 359-362
pMT302	hMT-IIA (mutant)	Cd ⁺⁺ , Zn ⁺⁺ , PMA ⁺⁺	Makarov, S.S., Jonat, C., and Haskill, S. (1994) Hyperinducible human metallothionein

			promoter with a low level basal activity. <i>Nucleic Acids Res.</i> 22 1504-1505
pIPF	hIFN- α , (interferon α)	Virus	Mori, T., Yamamoto, K., Ohta, T., Sakamoto, C., Sato, M., Koide, K., Murakami, T., Fujii, M., Fukuda, S., and Kurimoto, M. (1994) A high level and regulatable production system for recombinant glycoproteins using a human interferon - α promoter-based expression vector. <i>Gene</i> 144-289-293
pGRE5	5XGRE/Ad2MLP “(glucocorticoid reponse element/adenovirus major late promoter”)	Dexamethasone	Mader, S., and White, J.H. (1993) A steroid-inducible promoter for the controlled overexpression of cloned genes in eukaryotic cells.
GRE5	“high affinity glucocorticoid reponse element (GRE)/Adenovirus 2MLP”	Dexamethasone	S.Mader and White, A steroid-inducible promoter for the controlled overexpression of clonec genes in eukaryotic cells. <i>Proc.</i>

			<i>Natl. Acad. Sci. USA</i> 90 (1993), pp.5603-5607 0(12) 5603-7
pRDB	“DRE/MMTV (dioxin reponse element)”	TTCD’	.A.De Benedetti and R.E. Rhoads, A novel BK virus-based episomal vector for expression of foreign genes in mammalian cells. <i>Nucleic Acids Res.</i> 19 (1991), pp.1925-1931.

In a further aspect, the invention relates to a vector comprising a nucleic acid according to the present invention.

- 5 In a further aspect, the invention relates to a host cell which stably or transiently expresses a chimeric polypeptide, comprising:
 - a nucleic acid according to the present invention, which comprises or consists of a polynucleotide encoding the chimeric polypeptide, or a vector comprising said nucleic acid.
- 10 Preferably, the host cell further comprises:
 - an expression plasmid encoding a target polynucleotide of interest.

By the term vector or plasmid in the sense of the present invention, it is meant a DNA or RNA, circular or linear molecule, which is indifferently in the form of a single or double
15 strand.

A recombinant vector according the invention is preferably an expression vector. It may be a vector of bacterial or viral origin.

In any case, the nucleic acid encoding the polypeptide or the chimeric polypeptide of the invention is under the control of one or several sequences containing regulating signals of its expression in cells.

The choice of expression control sequence and expression vectors will depend upon the choice of host. A wide variety of expression host/vector combinations may be employed.

The preferred bacterial vectors according for the invention may be for example the vectors pBR322 (ATCC N 37017) or else the vectors such as pAA223-3 (Pharmacia, Uppsala, Sweden) and pGEM1 (PromegaBiotech, Madison, WI, USA). Other commercialised vectors may be cited, such as the vectors pQE70, qQE60, Pqe9 (QUIAGEN), psiX174, pBluescript SA, pNH8A, pMH16A, pMH18A, pMH46A, pWLNEO, pSV2CAT, pOG44, pXTI and pSG (Stratagene).

Useful expression vectors for eukaryotic hosts, include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from *E. coli*, including col E1, pCR1, pBR322, pMB9 and their derivatives, wider host range plasmids, such as M13 and filamentous single stranded DNA phages. Preferred *E. coli* vectors include pL vectors containing the lambda phage pL promoter (U.S. Pat. No. 4,874,702), pET vectors containing the T7 polymerase promoter (Studier et al., *Methods in Enzymology* 185: 60-89, 1990) and the pSP72 vector (Kaelin et al., *supra*). Useful expression vectors for yeast cells include the 2.mu. plasmid and derivatives thereof. Useful vectors for insect cells include pVL 941. Useful expression vectors for plant cells include, but are not limited to, cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV). In addition, any of a wide variety of expression control sequences may be used in these vectors. Such useful expression control sequences include the expression control sequences associated with structural genes of the foregoing expression vectors. Examples of useful expression control sequences include, for example, the early and late promoters of SV40 or adenovirus, the lac system, the trp system, the TAC or TRC system, the major operator and promoter

regions of phage lambda, for example pL, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast .alpha.-mating system and other sequences known to control the expression of genes or prokaryotic or eukaryotic cells
5 and their viruses, and various combinations thereof.

Any suitable host may be used to produce in quantity the polypeptides of the present invention described herein, including bacteria, fungi (including yeasts), plant, insect, mammal, or other appropriate animal cells or cell lines, as well as transgenic animals or plants. More particularly, these hosts may include well known eukaryotic and
10 prokaryotic hosts, such as strains of E.coli, Pseudomonas, Bacillus, Streptomyces, fungi, yeast, insect cells such as Spodoptera Frugiperda (SF9), and animal cells such as Chinese hamster ovary (CHO), mouse NS/O cells, African green monkey cells such as COS1, COS 7, BSC 1, BSC 40, and BMT 10, and human cells such as human embryonic kidney cells (HEK 293), as well as plant cells in tissue culture.

15 It should of course be understood that not all vectors and expression control sequences will function equally well to express the polypeptide or the polypeptidic complex of the present invention. Neither will all hosts function equally well with the same expression system. However, one of skill in the art may make a selection among these vectors, expression control systems and hosts without undue experimentation.

20 Any of the methods known in the art for the insertion of polynucleotide sequences into a vector may be used. See, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1989) and Ausubel et al., *Current Protocols in Molecular Biology*, J. Wiley & Sons, NY (1992), both of which are incorporated herein by reference. Conventional vectors consist of
25 appropriate transcriptional/translational control signals operatively linked to the polynucleotide sequence for producing the polypeptide or the polypeptidic complex of the present invention. Promoters/enhancers may also be used to control expression of polypeptide or the polypeptidic complex of the present invention. Promoter activation

may be tissue specific or inducible by a metabolic product or administered substance. Such promoters/enhancers include, but are not limited to, the native E2F promoter, the cytomegalovirus immediate-early promoter/enhancer (Karasuyama et al., *J. Exp. Med.*, 169: 13 (1989)); the human beta-actin promoter (Gunning et al., *Proc. Nat. Acad. Sci. USA*, 84: 4831 (1987)); the glucocorticoid-inducible promoter present in the mouse mammary tumor virus long terminal repeat (MMTV LTR) (Klessig et al., *Mol. Cell. Biol.*, 4: 1354 (1984)); the long terminal repeat sequences of Moloney murine leukemia virus (MuLV LTR) (Weiss et al., *RNA Tumor Viruses*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1985)); the SV40 early region promoter (Bernoist and Chambon, *Nature*, 290:304 (1981)); the promoter of the Rous sarcoma virus (RSV) (Yamamoto et al., *Cell*, 22:787 (1980)); the herpes simplex virus (HSV) thymidine kinase promoter (Wagner et al., *Proc. Nat. Acad. Sci. USA*, 78: 1441 (1981)); the adenovirus promoter (Yamada et al., *Proc. Nat. Acad. Sci. USA*, 82: 3567 (1985)).

Expression vectors compatible with mammalian host cells include, for example, plasmids; avian, murine and human retroviral vectors; adenovirus vectors; herpes viral vectors; and non-replicative pox viruses. In particular, replication-defective recombinant viruses can be generated in packaging cell lines that produce only replication-defective viruses. See *Current Protocols in Molecular Biology: Sections 9.10-9.14* (Ausubel et al., eds.), Greene Publishing Associates, 1989.

Specific viral vectors for use in gene transfer systems are now well established. See for example: Madzak et al., *J. Gen. Virol.*, 73: 1533-36 (1992: papovavirus SV40); Berkner et al., *Curr. Top. Microbiol. Immunol.*, 158: 39-61 (1992: adenovirus); Moss et al., *Curr. Top. Microbiol. Immunol.*, 158: 25-38 (1992: vaccinia virus); Muzyczka, *Curr. Top. Microbiol. Immunol.*, 158: 97-123 (1992: adeno-associated virus); Margulskes, *Curr. Top. Microbiol. Immunol.*, 158: 67-93 (1992: herpes simplex virus (HSV) and Epstein-Barr virus (EBV)); Miller, *Curr. Top. Microbiol. Immunol.*, 158: 1-24 (1992: retrovirus); Brandyopadhyay et al., *Mol. Cell. Biol.*, 4: 749-754 (1984: retrovirus); Miller

et al., *Nature*, 357: 455-450 (1992: retrovirus); Anderson, *Science*, 256: 808-813 (1992: retrovirus), all of which are incorporated herein by reference.

Nucleic acids encoding the polypeptide of the invention can be introduced into eukaryotic cells growing in culture in vitro by conventional transfection techniques (e.g., calcium phosphate precipitation, DEAE-dextran transfection, electroporation, lipofection, etc.).
5 Nucleic acid can also be transferred into cells in vivo, for example by application of a delivery mechanism suitable for introduction of nucleic acid into cells in vivo, such as retroviral vectors (see e.g., Ferry, N et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:8377-8381; and Kay, M. A. et al. (1992) *Human Gene Therapy* 3:641-647), adenoviral vectors
10 (see e.g., Rosenfeld, M. A. (1992) *Cell* 68:143-155; and Herz, J. and Gerard, R. D. (1993) *Proc. Natl. Acad. Sci. USA* 90:2812-2816), receptor-mediated DNA uptake (see e.g., Wu, G. and Wu, C. H. (1988) *J. Biol. Chem.* 263:14621; Wilson et al. (1992) *J. Biol. Chem.* 267:963-967; and U.S. Pat. No. 5,166,320), direct injection of DNA (see
15 e.g., Acsadi et al. (1991) *Nature* 332:815-818; and Wolff et al. (1990) *Science* 247:1465-1468) or particle bombardment (see e.g., Cheng, L. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:4455-4459; and Zelenin, A. V. et al. (1993) *FEBS Letters* 315:29-32).

Appropriate culture media to be used for cultivating recombinant host cells and production of recombinant proteins are well known from the man skilled in the art.

20 In a further aspect, the invention relates to a protein translation control system of a target polynucleotide of interest comprising a chimeric polypeptide according the present invention, or a nucleic acid according to the present invention, which comprises or consists of a polynucleotide encoding the chimeric polypeptide, or a vector comprising said nucleic acid, or a host cell according to the present invention. Preferably, the protein
25 translation control system further comprises an expression plasmid encoding a target polynucleotide of interest.

In a further aspect, the invention relates to a method for controlling *in vitro* the translation of a target polynucleotide of interest, which comprises the following steps:

- (a) introducing in a host cell the target polynucleotide of interest;
- (b) cultivating the recombinant host cell obtained at step (a) in an appropriate culture medium, thus allowing said host cell to stably express said target polynucleotide of interest; and
- (c) adding to the culture medium a chimeric polypeptide according to the present invention in order to activate the expression of said target polynucleotide of interest.

10 In a further aspect, the invention relates to a method for controlling *in vitro* the translation of a target polynucleotide of interest, which comprises the following steps:

- (a) introducing in a host cell the target polynucleotide of interest as well as a nucleic acid according to the present invention, which comprises or consists of a polynucleotide encoding the chimeric polypeptide, or a vector comprising said nucleic acid;
- (b) cultivating the recombinant host cell obtained at step (a) in an appropriate culture medium, thus allowing said host cell to stably express said target polynucleotide of interest; and
- (c) adding to the culture medium an agent capable to interact with the inducible regulatory polynucleotide, thus allowing to regulate the expression of the nucleic acid encoding the chimeric polypeptide.

In a further aspect, the invention relates to a kit for controlling the translation of a target polynucleotide of interest, comprising a chimeric polypeptide according the present invention, or a nucleic acid according to the present invention, which comprises or consists of a polynucleotide encoding the chimeric polypeptide, or a vector comprising said nucleic acid.

Preferably, the kit further comprises a recombinant vector comprising the target polynucleotide of interest.

In a further aspect, the invention relates to a kit for controlling the translation of a target polynucleotide of interest, comprising a host cell according to the present invention. Preferably, said kit further comprises a recombinant vector comprising the target polynucleotide of interest.

Kits typically comprise two or more components necessary for performing said detection or for performing a diagnostic assay. Components may be compounds, reagents, containers and/or equipment.

In a further aspect, the invention relates to the use of a protein translation control system according to the present invention or of a kit according to the present invention for activating the translation of a target polynucleotide of interest in a bioreactor comprising host cells in culture.

The invention also relates to a method for the production of a polypeptide according to the invention or a chimeric polypeptide according to the invention, comprising the following steps:

- a) inserting a nucleic acid encoding the polypeptide in an appropriate expression vector,
- b) cultivating, in an appropriate culture medium, a host cell previously transformed or transfected with the recombinant vector obtained at step (a),
- c) recovering the conditioned culture medium or lyse the host cell, for example by sonication or by osmotic change,
- d) separating and purifying, from the culture medium, or else from the cell lysates obtained at step (c), said polypeptide,

e) optionally, characterising the produced recombinant polypeptide.

The polypeptides of the invention may be characterised by fixation on an immunoaffinity chromatography column on which antibodies directed to the polypeptide have been first
5 immobilised.

In another aspect, a polypeptide according to the invention or a chimeric polypeptide according to the invention, may be purified by migration in a series of chromatography columns, according to well known methods, described in AUSUBEL et al. (1989,
10 Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience N. Y.). Said polypeptide may also be prepared by the classical techniques of chemical synthesis, in homogeneous solution or in solid phase. As an illustrative example, a polypeptide according to the invention or a chimeric polypeptide according to the invention, may be prepared by the technique in homogeneous solution disclosed by
15 HOUBEN WEIL (1974, In methode der Organischen Chemie, E.Wunshed., volume 15-I et15-li, Thieme, Stuttgart) or else by the technique of synthesis in solid phase disclosed by MERRIFIELD (1965a, Nature, vol. 207 (996) : 522-523; 1965b Science, vol. 150 (693) : 178-185).

20 Lastly, the invention relates to a pharmaceutical composition comprising a chimeric polypeptide according to the present invention.

Table 3: Identity scores for Rotavirus group A NSP3 proteins (33 sequences - major divergent sequences excluded)

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
	AAL 58537 Simian SA11-C14	AAV65739 Human Stain TB-Chen,serotype G2	YP338254 Human G3, strain B4106 serotype P141G3	ABC66304 Italian lapine 30/96 serotype G39 [14]	AAV55967 Human strain DRC86	AAV55956 Human strain DRC88	AAK74118 Human strain M clone M1	AAK74117 Human strain M clone M1	AAK74116 Human strain M clone M0	AAF81408 Human strain IGV-F	AAF81407 Human strain IGV-S	AAF81406 Human strain IGV-P	BAA84968 Human strain KU	AAL58536 Rhesus	AAQ07950 Human strain RMC100	CAA54093 Human strain IS2	CAA57193 Human strain WA (Rotavirus G ₉ , serotype 1)	CAA57195 Human strain ST3 (Rotavirus G ₉ , serotype 4)	CAA57187 Human strain S2	CAA57184 Human strain 69M (Rotavirus G ₉ , serotype 8)	CAA57194 Human strain KU (Rotavirus G ₉ , serotype 1)	CAA57196 Human strain w161 (Rotavirus G ₉ , serotype 9)	CAA57192 Human strain 1231	CAA57186 Human(Rotavirus hg17)	AAQ07954 Human strain NR1	AAD38365 Human strain MP409	CAA57185 Human stain rrv (Rotavirus G ₉ , serotype 3)	AAQ11370 Human strain RMC321	AAU43791 Human rotavirus G3 strain B4106, serotype P141, G3	CAA57189 Porcine rotavirus, strain G0 (Rotavirus G serotype 4)	CAA57190 Porcine rotavirus strain=OSU (Rotavirus G serotype 4)	CAA57191 Porcine rotavirus, strain PRICE (Rotavirus G serotype 3)
1	CAA79754.1 Bovine strain RF	79	82	96	83	83	88	87	88	88	88	87	86	84	86	79	86	88	82	83	88	86	87	94	83	92	83	89	96	87	87	86
2	AAL 58537 Simian SA11-C14	\$	78	79	79	79	77	77	77	77	77	76	76	82	78	77	76	77	78	77	77	76	76	78	79	74	81	78	79	77	77	76
1	CAA79754.1 Bovine strain RF	75	77	96	77	77	85	84	85	85	85	84	85	79	83	75	83	86	76	78	86	84	86	95	77	90	77	89	96	86	85	83
2	AAL 58537 Simian SA11-C14	\$	77	75	78	78	73	73	73	73	73	72	72	80	73	77	72	73	77	79	72	72	72	75	78	68	78	77	75	74	73	72
1	CAA79754.1 Bovine strain RF	70	73	94	73	73	83	82	83	83	82	83	73	73	81	72	82	84	73	73	84	83	85	95	73	87	71	87	94	84	85	82
2	AAL 58537 Simian SA11-C14	\$	75	68	75	75	67	67	67	67	66	66	74	74	68	75	67	67	67	75	66	66	67	70	75	88	72	72	69	68	69	67

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146-
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5 The invention is further embodied in the following examples and figures.

FIGURES

Figure 1. Cap-dependent translation.

10 The factor eIF4G (open ellipse) acts a platform on which the factor eIF4E (cross hatched), bound to the cap (black dot) at the 5' end of the mRNA, and the PABP (diagonal hatched), associated with the 3' poly(A) tail, can bind. The 40S and 60S ribosomal sub-units (grey ellipses), the initiation (AUG) and termination (STOP) codons are indicated (black squares).

15

Figure 2. General structure of a Cytomoduline.

Cytomodulines with both positive (Cytoboost) or negative (anti-viral) effects are depicted on the left or right respectively by ellipses. The tethering (cross hatched) and effector (white) domains are distinct. In principle, the same tethering domain can be used for both
20 positive and negative Cytomodulines. This possibility is used to test the effector domains.

Figure 3. Stimulation of translation by the prototype Cytomoduline.

Hela cells, were co-transfected with a plasmid encoding a Cytomoduline and a second plasmid from which two reporter mRNAs are transcribed that encode individually the
25 Renilla and Firefly Luciferases. The identity of the Cytomoduline expressed is indicated below the graph. In the reporter plasmid the reading frame encoding the Renilla Luciferase is followed by the MS2 stem loop sequence recognised by the MS2CP protein present in the Cytomodulines. The activity of the Firefly Luciferase allows a normalisation with respect to the transfection efficiency. The Cytomoduline
30 MS2CP/CUG-BP1, prototype of an inhibitory Cytomoduline is used here as a negative

control. The two bars for the MS2CPNSP3 Cytomoduline correspond to two separate experiments. In these experiments the positive Cytomoduline was encoded by a genetic in-frame fusion of two MS2-CP sequences followed by amino acids 163 to 313 of bovine NSP3 RF strain (see sequence SEQ ID N°48). The Luciferase activity is expressed as the ratio of Renilla to Firefly activities. The values given are the average \pm standard deviation for triplicate assays in an individual transfection experiments.

Figure 4. Stimulation of translation by the prototype Cytomoduline: comparison of the effect in HeLa and HEK 293 cells.

10 HeLa or HEK 293 cells, as indicated below the figure, were co-transfected with a plasmid encoding a Cytomoduline and a second plasmid from which two reporter mRNAs are transcribed that encode individually the Renilla and Firefly Luciferases. The identity of the Cytomoduline expressed is indicated on the right of the graph. The plasmids encoding the reporter mRNA and cytomoduline are the same as those described in the legend to figure 15 3. The Luciferase activity is expressed as the ratio of Renilla to Firefly activities (Luc R / Luc F) relative to this ratio observed for the Cytomoduline containing MS2CP alone. The values given are the average \pm standard deviation of data obtained in three separate experiments (n= 3).

20 **Figure 5. Schematic organisation of the peptides derived from the NSP3 protein of the group A rotavirus strain Simian SA11**

Figure 6. Schematic organisation of the peptides derived from the NSP3 protein of the group A bovine rotavirus strain RF

25

EXAMPLES

The inventors have obtained a positive Cytomoduline. This positive Cytomoduline is composed of a RNA-binding or tethering domain containing the phage protein MS2CP and an effector domain derived from the viral protein NSP3. In a first demonstration the effect of this Cytomoduline on the expression of a reporter protein was studied in HeLa cells, which is a standard established human cell line derived from a Human Negroid cervix epitheloid carcinoma. The effect of the positive Cytomoduline on protein expression in these cells was compared with that caused by the presence of a previously described negative Cytomoduline derived from the same tethering protein (MS2-CP) fused with the human protein CUG-BP1 (Peptide protein translation inhibitor and the use thereof for protein translational control, WO2005010038 – 2005-02-03). As a further control the effect of expressing the tethering protein alone (fused with a C-terminal HA tag) on reporter protein expression was also determined. The mRNA encoding the reporter protein (Renilla Luciferase) was transcribed from a bidirectional CMV promoter within a transfected plasmid. In this mRNA the 3' untranslated regions that followed the Renilla Luciferase coding region, contained multiple binding sites for the tethering protein. The gene encoding the Firefly Luciferase was also expressed from this same bidirectional CMV promoter. This mRNA was devoid of binding sites for the tethering protein and hence allowed normalisation of the transfection efficiency. Accordingly, the data is expressed as the ratio of Renilla to Firefly luciferase activities (Luc R/Luc F).

In figure 3 are shown the data obtained for two separate transfections of HeLa cells with the positive Cytomoduline (MS2CP/NSP3), for cells transfected with the negative Cytomoduline MS2-CP/CUG-BP1 and for cells transfected with the HA tagged form of MS2CP. Taking the normalised expression of Renilla Luciferase in the presence of HA tagged MS2CP as a base line, the expression on Renilla Luciferase decreased in the presence of the negative Cytomoduline as would be expected. In contrast, the expression

of Renilla Luciferase was strongly enhanced in cells that also expressed the positive Cytomoduline (MS2CP/NSP3).

To ensure that this stimulatory effect of the positive Cytomoduline was not restricted to
5 HeLa cells, these measurements were repeated using HEK 293 cells. HEK 293 cells also
have an epithelial morphology and originated from a Human Embryo Kidney. The results
for this second demonstration are given in figure 4 where the data for the HEK 293 cells
is compared with that from HeLa cells. The data for the HeLa cells shown in figure 4 are
distinct from those shown in figure 3 and result from new experiments. For both cell
10 types, three independent transfections were made of each the effector plasmids. Again,
the expected decrease of Renilla Luciferase activity was observed when the negative
Cytomoduline was expressed in either cell type. Furthermore, the positive Cytomoduline
also stimulated the expression of Renilla Luciferase in HEK 293 cells showing that the
effect is not restricted to a single cell type.

15

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CLAIMS

1. A polypeptide comprising the amino-acid sequence SEQ ID N°32 or a sequence
5 having at least 70 % of identity with SEQ ID N°32, or any one of its fragments, which is
capable of activating protein translation.
2. The polypeptide according to claim 1, wherein the fragment is selected from the
group consisting of the fragments of sequence SEQ ID N°33 and sequences having at
10 least 70 % of identity with SEQ ID N°33.
3. The polypeptide according to claim 1, wherein the fragment is selected from the
group consisting of the fragments of sequence SEQ ID N°34, SEQ ID N°35 and
sequences having at least 70 % of identity with SEQ ID N°34 and SEQ ID N°35.
15
4. The polypeptide according to claim 1, wherein the fragment is selected from the
group consisting of the fragments of sequence SEQ ID N°36, SEQ ID N°37 and
sequences having at least 70 % of identity with SEQ ID N°36 and SEQ ID N°37.
- 20 5. The polypeptide according to claim 1, wherein the fragment is selected from the
group consisting of the fragments of sequence SEQ ID N°38, SEQ ID N°39 and
sequences having at least 70 % of identity with SEQ ID N°38 and SEQ ID N°39.
6. The polypeptide according to claim 1, wherein the fragment is selected from the
25 group consisting of the fragments of sequence SEQ ID N°40, SEQ ID N°41 and
sequences having at least 70 % of identity with SEQ ID N°40 and SEQ ID N°41.

7. The polypeptide according to claim 1, wherein the fragment is selected from the group consisting of the fragments of sequence SEQ ID N°42, SEQ ID N°43 and sequences having at least 70 % of identity with SEQ ID N°42 and SEQ ID N°43.
- 5 8. The polypeptide according to claim 1, wherein the fragment is selected from the group consisting of the fragments of sequence SEQ ID N°44, SEQ ID N°45 and sequences having at least 70 % of identity with SEQ ID N°44 and SEQ ID N°45.
9. The polypeptide according to anyone of claims 1 to 8, wherein the sequences
10 having at least 70 % of identity with anyone of sequences SEQ ID N°32 to SEQ ID N°45 originate from anyone of the following rotaviruses : bovine rotavirus A, caprine rotavirus A, equine rotavirus A, human rotavirus A, lapine rotavirus, porcine rotavirus A, rabbit rotavirus, rotavirus 5 serotype G2, rotavirus G8, rotavirus subgroup 1, rotavirus subgroup 2, simian rotavirus A/SA11, simian rotavirus A/SA11-C14 and swine rotavirus
15 strain S8.
10. A chimeric polypeptide capable of specifically activating the translation of a target polynucleotide of interest, which comprises the Non-Structural Protein 3 (NSP3) of sequence SEQ ID N°46 or a sequence having at least 70 % of identity with SEQ ID N°46
20 or anyone of the fragments thereof, wherein said NSP3 or anyone of its fragments is fused to a RNA binding protein.
11. The chimeric polypeptide according to claim 10, wherein the fragment of the NSP3 protein is the polypeptide of anyone of claims 1 to 9.
25
12. The chimeric polypeptide according to claim 10, whose sequence comprises the sequence SEQ ID N°48.

13. The chimeric polypeptide according to anyone of claims 10 to 12, wherein the RNA binding protein is selected from the group consisting of MS2CP, N, IRP and U1A.

14. The chimeric polypeptide according to anyone of claims 10 to 13, which further
5 comprises a transport signal which allows the chimeric polypeptide to cross the plasma membrane of the target cells.

15. A nucleic acid comprising a polynucleotide encoding a polypeptide according to anyone of claims 1 to 9.

10

16. A nucleic acid comprising a polynucleotide encoding a chimeric polypeptide according to anyone of claims 10 to 14.

15

17. The nucleic acid of claim 16, comprising the polynucleotide of sequence SEQ ID N°49.

18. The nucleic acid according to claim 16 or 17, which further comprises an inducible regulatory polynucleotide for the control of the polynucleotide encoding the chimeric polypeptide according to anyone of claims 10 to 14.

20

19. A vector comprising a nucleic acid according to anyone of claims 16 to 18.

20. A host cell which stably or transiently expresses a chimeric polypeptide, comprising:

25

- a nucleic acid according to anyone of claims 16 to 18 or a vector according to claim 19.

21. A host cell according to claim 20, further comprising:

- an expression plasmid encoding a target polynucleotide of interest.

22. A protein translation control system of a target polynucleotide of interest
5 comprising a chimeric polypeptide according to anyone of claims 10 to 14, or a nucleic acid according to anyone of claims 16 to 18, or a vector according to claim 19, or a host cell according to anyone of claims 20 to 21.

23. A method for *in vitro* controlling the translation of a target polynucleotide of
10 interest, which comprises the following steps:

- (a) introducing in a host cell the target polynucleotide of interest;
- (b) cultivating the recombinant host cell obtained at step (a) in an appropriate culture medium, thus allowing said host cell to stably express said target polynucleotide of interest; and
- 15 (c) adding to the culture medium a chimeric polypeptide according to anyone of claims 10 to 14 in order to activate the expression of said target polynucleotide of interest.

24. The method of claim 23, wherein the chimeric polypeptide further comprises a
20 transport signal which allows said chimeric polypeptide to cross the plasma membrane of the target cells.

25. A method for *in vitro* controlling the translation of a target polynucleotide of interest, which comprises the following steps:

- 25 (a) introducing in a host cell the target polynucleotide of interest as well as a nucleic acid according to claim 18 or a vector according to claim 19 comprising said nucleic acid;

(b) cultivating the recombinant host cell obtained at step (a) in an appropriate culture medium, thus allowing said host cell to stably express said target polynucleotide of interest; and

(c) adding to the culture medium an agent capable to interact with the
5 inducible regulatory polynucleotide, thus allowing to regulate the expression of the nucleic acid encoding the chimeric polypeptide.

26. A kit for controlling the translation of a target polynucleotide of interest, comprising a chimeric polypeptide according to anyone of claims 10 to 14 or a vector
10 according to claim 19.

27. The kit according to claim 26, further comprising a recombinant vector comprising the target polynucleotide of interest.

15 28. A kit for controlling the translation of a target polynucleotide of interest, comprising a host cell according to anyone of claims 20 to 21.

29. Use of a protein translation control system according to claim 22 or a kit according to anyone of claims 26 to 28 for activating the translation of a target polynucleotide of
20 interest in a bioreactor comprising host cells in culture.

30. A pharmaceutical composition comprising a chimeric polypeptide according to anyone of claims 10 to 14.

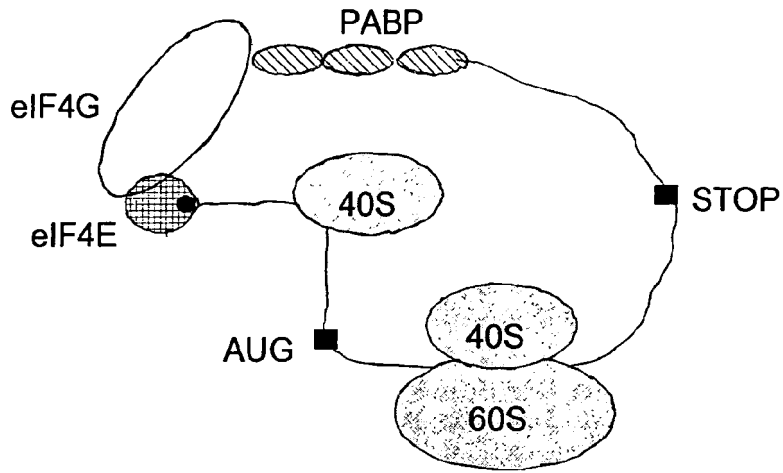


FIGURE 1

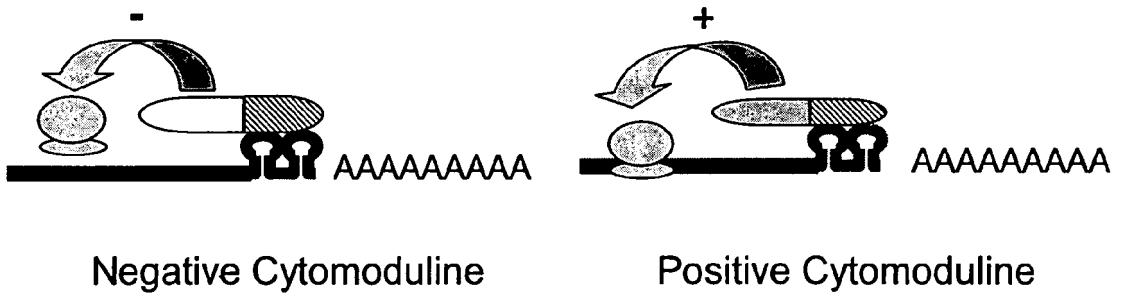


FIGURE 2

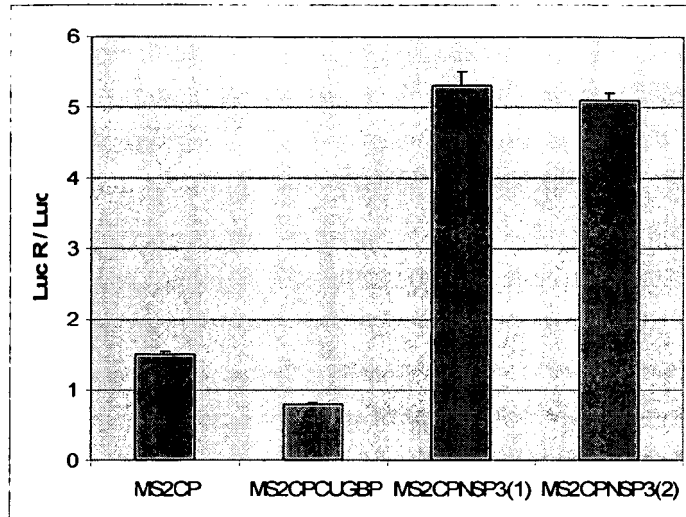


FIGURE 3

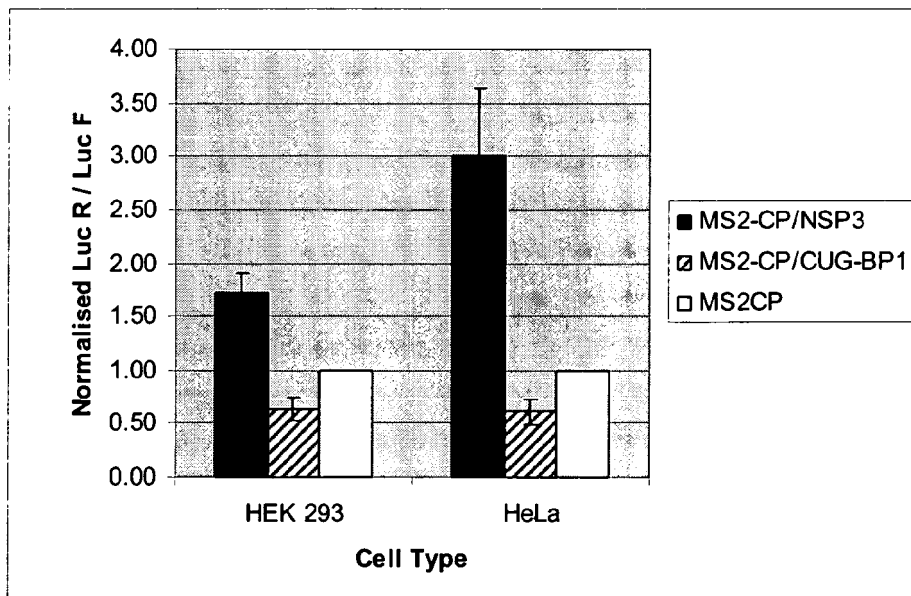


FIGURE 4

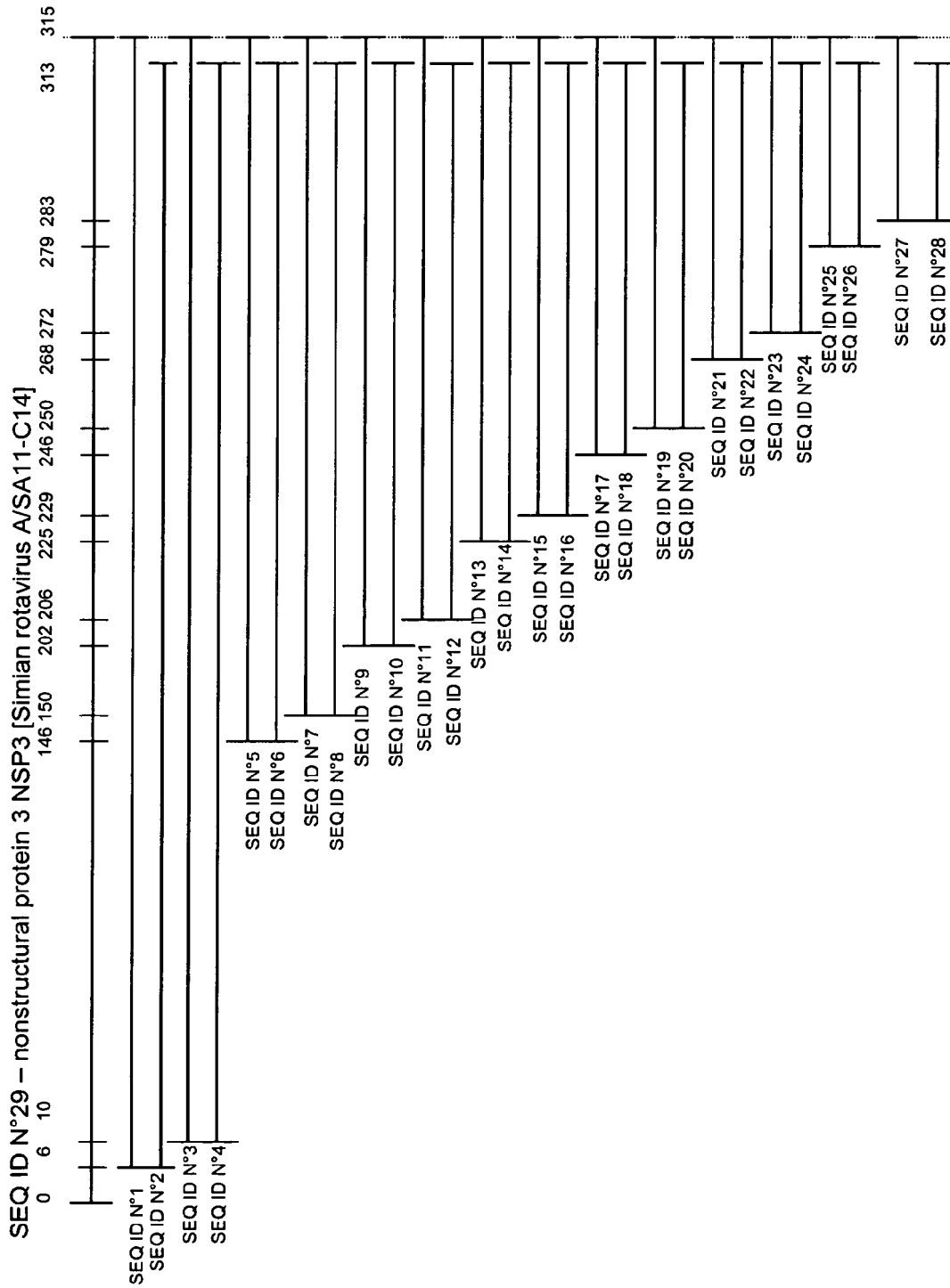


FIGURE 5

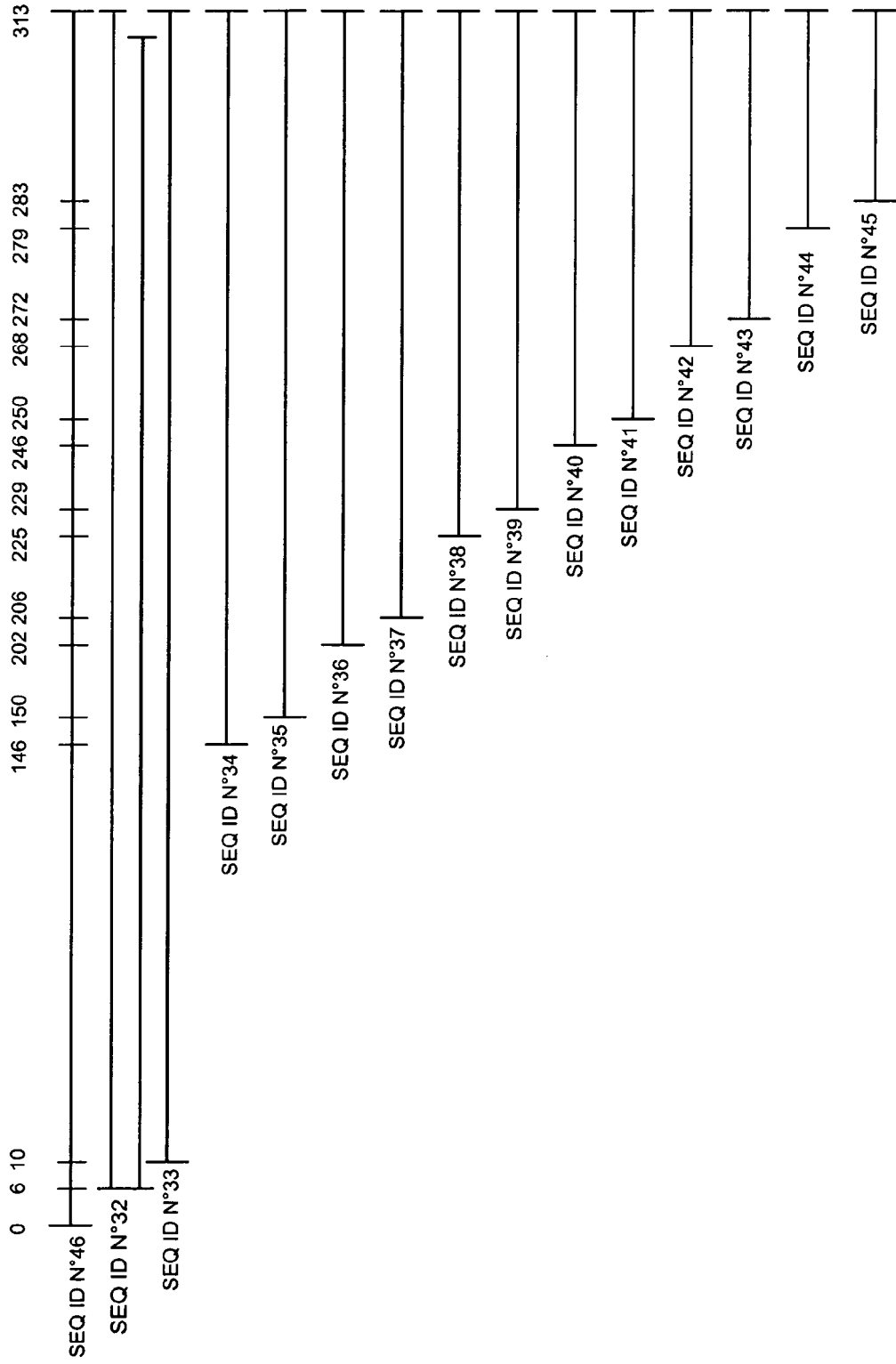


FIGURE 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2007/057483

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C07K14/14 C12N15/67 C07K19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
 EPO-Internal, BIOSIS, WPI Data, Sequence Search, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	APONTE C ET AL: "Expression of two bovine rotavirus non-structural protein (NSP2, NSP3) in the baculovirus system and production of monoclonal antibodies directed against the expressed proteins" ARCHIVES OF VIROLOGY, vol. 133, no. 1-2, 1993, pages 85-95, XP009092613 ISSN: 0304-8608 page 87, last paragraph - page 88, paragraph 1 ----- -/--	1-9, 15

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family
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Date of the actual completion of the international search 29 November 2007	Date of mailing of the international search report 12/12/2007
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Huber, Angelika
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2007/057483

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>RAO ET AL: "Comparative nucleotide and amino acid sequence analysis of the sequence specific RNA-binding rotavirus nonstructural protein NSP3" VIROLOGY, ACADEMIC PRESS, ORLANDO, US, vol. 207, 1995, pages 327-333, XP002987916 ISSN: 0042-6822 abstract; figures 1,2</p> <p>-----</p>	1-9,15
X	<p>MAUNULA LEENA ET AL: "Frequent reassortments may explain the genetic heterogeneity of rotaviruses: analysis of Finnish rotavirus strains." JOURNAL OF VIROLOGY DEC 2002, vol. 76, no. 23, December 2002 (2002-12), pages 11793-11800, XP002460431 ISSN: 0022-538X page 11795, left-hand column, last paragraph & DATABASE UniProt [Online] 1 October 2000 (2000-10-01), "Non-structural protein 3 (Fragment)." retrieved from EBI accession no. UNIPROT:Q9IVH9 Database accession no. Q9IVH9</p> <p>-----</p>	1,5,9,15
X	<p>WO 02/38738 A (COLD SPRING HARBOR LAB [US]; KRAINER ADRIAN R [US]; CARTEGNI LUCA [US]) 16 May 2002 (2002-05-16) page 24, lines 1-25</p> <p>-----</p>	10,11, 16,22
Y	<p>WO 00/53779 A (EUROP MOLECULAR BIOLOGY LAB [DE]; HENTZE MATTHIAS W [DE]; GREGORIO ENN) 14 September 2000 (2000-09-14) page 8, line 34 - page 9, line 6 page 9, lines 15-20</p> <p>-----</p>	10-30
Y	<p>WO 2005/010038 A (CENTRE NAT RECH SCIENT [FR]; UNIV RENNES [FR]; COSSON BERTRAND [FR]; P) 3 February 2005 (2005-02-03) page 3, lines 15-31 page 16, lines 6-34; claims 27-40; table 1</p> <p>-----</p>	10-30
A	<p>PIRON MARIA ET AL: "Identification of the RNA-binding, dimerization, and eIF4GI-binding domains of rotavirus nonstructural protein NSP3" JOURNAL OF VIROLOGY, vol. 73, no. 7, July 1999 (1999-07), pages 5411-5421, XP002460432 ISSN: 0022-538X cited in the application the whole document</p> <p>-----</p>	10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2007/057483

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			DE 602004003835 T2 09-08-2007
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