RTMP'S COPY

9703541-4

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/12, C07K 14/47, C12N 5/10, A61K 38/17 — A1

(11) International Publication Number: WO 96/25492

(43) International Publication Date: 22 August 1996 (22.08.96)

(21) International Application Number: PCT/EP96/00585

(22) International Filing Date: 12 February 1996 (12.02.96)

(30) Priority Data:
9502771.0 13 February 1995 (13.02.95) GB
9513505.9 3 July 1995 (03.07.95) GB

(71) Applicant *(for all designated States except AT DE US)*: SANDOZ LTD. [CH/CH]; Lichtstrasse 35, CH-4002 Basle (CH).

(71) Applicant *(for DE only)*: SANDOZ-PATENT-GMBH [DE/DE]; Humboldtstrasse 3, D-79539 Lörrach (DE).

(71) Applicant *(for AT only)*: SANDOZ-ERFINDUNGEN VERWALTUNGSGESELLSCHAFT MBH [AT/AT]; Brunner Strasse 59, A-1230 Vienna (AT).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: HAUBER, Joachim [DE/AT]; Nothartgasse 21/10, A-1130 Vienna (AT).

(74) Common Representative: SANDOZ LTD.; Patents & Trademarks Div., Lichtstrasse 35, CH-4002 Basle (CH).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: EUKARYOTIC INITIATION FACTOR 5A (eIF-5A) MUTANTS

(57) Abstract

eIF-5A mutant proteins which are normally devoid of substantial eIF-5A activity and which inhibit Rev function and thus possess an inhibitory phenotype with respect to Rev function, and genes or DNA or cDNA sequences coding therefor.

EUKARYOTIC INITIATION FACTOR 5A (eIF-5A) MUTANTS

Human Immunodeficiency Virus (HIV) replication depends critically on the function of the viral Rev trans-activator protein. Rev accumulates in the nuclear compartment of expressing cells and binds directly and specifically a highly structured RNA sequence, termed Rev Response Element (RRE), which is present on all incompletely-spliced viral mRNAs. After binding, Rev appears to multimerize on the RRE, and it interacts with a host co-factor required for Rev transactivation, named eukaryotic initiation factor 5A (eIF-5A; older terminology: eIF-4D). This interaction results in nucleo-/cytoplasmic translocation of the incompletely-spliced viral mRNAs, subsequently allowing the expression of the viral structural proteins (e.g. Gag, Pol, Env), and hence viral replication.

Previously, Rev mutant proteins with trans-dominant (dominant negative) phenotypes have been described (see e.g. WO 90/14427). These Rev mutants are still able to bind the RRE RNA but are incapable of interacting functionally with their cellular co-factor due to a mutation in the Rev activation (effector) domain.

eIF-5A is an endogenous protein needed for normal cellular function, several variant forms thereof have been prepared (e.g. J.Biol.Chem. 264 [1989] 18527-18530; Mol.Cell.Biol. 11 [1991] 3105-3114), and these appear to have unfavourable effects on normal cellular function, such as cell growth.

It has now been found that mutant eIF-5A proteins can be produced which do not block endogenous wild-type eIF-5A function and, normally, are themselves devoid of any significant eIF-5A activity, but which inhibit Rev function: they possess an inhibitory phenotype with respect to Rev function.

This is very surprising, considering that it would not be expected that a factor which is inactive in the normal cellular environment of its wild-type form, would nevertheless have an inhibitory effect on metabolic events initiated by an extraneous organism, such as a virus. It was not to be expected that such mutant eIF-5A proteins

and genes coding therefor could exist at all due to the fact that eIF-5A is a protein which is essential for cell regulation (Biofactors 4 [1993] 95-104; TIBS 18 [1993] 475-479). It was expected that such genes and proteins would be toxic to the cell, whereas the genes and proteins claimed with the present invention (in particular, specifically, M13, M14 and M13Δ) are normally inactive, non-toxic, and inhibitory as regards Rev, e.g. HIV Rev function and therefore HIV replication.

The present invention concerns eIF-5A mutant proteins which normally have lost their normal cellular eIF-5A activity (herein defined as endogenous eIF-5A function), but which inhibit Rev function and thus possess an inhibitory phenotype with respect to Rev function; and corresponding genes or DNA or cDNA sequences coding therefor.

Preferred are mutants which are themselves devoid of substantial endogenous eIF-5A function. eIF-5A as used herein preferably is human eIF-5A, and the mutants preferably are based on the human wild-type version of eIF-5A. HIV preferably is HIV-1.

The mutant proteins of the present invention should be understood as polypeptides including variants such as allelic variants, or fragments or derivatives which still have an inhibitory effect on Rev function, while being themselves preferably devoid of substantial endogenous eIF-5A function. For pharmacological applications they are preferably in substantially pure form, e.g. substantially free of products from their endogenous cellular environment.

The mutation or mutations preferably involve a region or regions on the polypeptide starting from about amino acid position 130 of the wild-type eIF-5A genome (Figure 3; SEQ ID No. 17), especially between about amino acid position 135 and the end of the molecule, particularly between positions 135 and 139.

The corresponding genes or DNA or cDNA sequences are e.g. as set out in Table 2 in particular for mutants eIF-5AM13, eIF-5AM14 and eIF-5AM13Δ, or are genes or sequences which hybridize under stringent conditions to the protein coding regions thereof, or are genes or sequences which, but for the degeneracy of the genetic code, would hybridize therewith, while preferably not hybridizing with the genes or sequences for the corresponding wild-type forms.

The invention also includes prokaryotic or eukaryotic host cells transformed or transfected with a gene or a DNA or cDNA sequence as defined above in a manner allowing the host cell to express the gene or the DNA or cDNA sequence. It further concerns biologically functional plasmid or viral DNA vectors including a gene or a DNA or cDNA sequence as defined above. It also concerns prokaryotic or eukaryotic host cells stably transformed or transfected with a DNA vector as defined above.

The invention also concerns a process for the preparation of genes or DNA or cDNA sequences as defined above, comprising isolating the corresponding wild-type gene from an appropriate expression system, inserting this gene into an appropriate cloning system, introducing the desired mutation into the gene and recovering the resultant mutant from the clones having the desired mutation.

It further includes a process for the production of proteins as defined above, which comprises culturing under suitable nutrient conditions a prokaryotic or eukaryotic host cell transformed or transfected with a gene or a DNA or cDNA sequence as defined above in a manner alllowing the host cell to express the protein, and optionally isolating the desired polypeptide product of the expression.

It further concerns pharmaceutical compositions comprising proteins as defined above, together with at least one pharmaceutically acceptable carrier, adjuvant or diluent, or in the form of cells taken from a patient's body and treated in vitro prior to reinsertion. It also concerns the use of proteins as defined above or of genes or DNA or cDNA sequences coding therefor in the treatment of diseases caused by retroviruses dependent on eIF-5A for Rev function, such as HIV, HTLV-I and/or HTLV-II. It also concerns their use as a pharmaceutical, and their use in the preparation of a medicament for the treatment of diseases caused by retroviruses dependent on eIF-5A for Rev function, such as HIV, HTLV-I and/or HTLV-II.

The procedures and techniques to be used in employing the present invention are known in the art. Insofar as their preparation is not particularly described herein, the compounds, reagents, vectors, cell-lines, etc. to be used for carrying out the invention are known and publicly available or may be obtained in conventional manner from known and publicly available materials, or equivalent materials may be prepared in conventional manner from known and publicly available materials.

a) Generation of eIF-5A mutants

A human cDNA encoding eIF-5A (J. Cell Biol. 123/6, 1309-1320 [1993]) was cloned as HindIII fragment in the HindIII site of the M13mp18 vector (Pharmacia, Sweden). The corresponding single-stranded DNA was used for mutagenesis employing the "Oligonucleotide-directed in vitro mutagenesis system, Version 2" (Amersham, UK). The sequence of all mutants was determined by DNA sequencing employing Sequenase 2.0 from United States Biochemicals. Subsequently, all eIF-5A mutant genes (see Table I) were expressed in COS cells and subjected to eIF-5A-specific immunoprecipitation analysis in order to confirm protein expression.

Table 1
eIF-5A mutants

Construct	Position (aa)	Mutation (aa)	Rev *in vitro* binding	Complementation in yeast
peIF-5AM1	4,5,6	D,L,D→E,D,L	nd	yes
peIF-5AM2	9,10	T,G→D,L	nd	yes
peIF-5AM3	15,16,17	S,A,T→A,D,L	nd	yes
peIF-5AM4	22,23,24	C,S,A→G,D,L	-	no
peIF-5AM5	43,44,45	M,S,T→I,D,L	-	no
peIF-5AM6	46,47,48	S,K,T→L,D,L	-	no
peIF-5AM7	64,65	F,T→D,L	-	no
peIF-5AM8	69,70	Y,E→D,L	-	no
peIF-5AM9	75,76	S,T→D,L	-	no
peIF-5AM10	98,99,100	Y,L,S→L,D,L	-	no
peIF-5AM11	104,105,106	D,S,G→Q,D,L	nd	yes
peIF-5AM12	126,127	K,Y→D,L	nd	yes
peIF-5AM13	135,136	I,T→D,L	+	no
peIF-5AM14	138,139	L,S,→D,L	+	no
peIF-5AM15	141,142,143	M,T,E→I,D,L	nd	yes
peIF-5AM16	149,150	I,K→D,L	-	no
peIF-5AM13Δ*	135	DLCCLP-STOP	+	no

*peIF-5AM13Δ is a 140 amino acid (aa) C-terminal eIF-5A deletion protein of following sequence: wild-type aa 1-134-DLCCLP. All other mutants are amino acid substitutions. +, wild-type activity; -, no activity. nd, not determined.

The nucleotide sequence of the specific oligonucleotides used for the production of the above 17 mutants is as appears hereunder (Table 2) (SEQ ID No. 1 to No. 16, respectively), whereby the last mutant, M13Δ, was obtained with the use of the same oligonucleotide as for M13: a mistake of the mutagenesis reaction resulted in M13Δ.

It should be appreciated that these specific nucleotide sequences are only illustrative for the 17 specific mutants produced and that identical mutants can be obtained with many different nucleotide sequences, considering e.g. wobbling of codon usage.

Table 2
Oligonucleotides for eIF-5A mutants

M1:	5'- GGC AGA TGA AGA TCT CTT CGA GAC AGG -3'
M2:	5'- CTT GGA CTT CGA AGA TCT AGA TGC AGG -3'
M3:	5'- GCA GGG GCC GCA GAT CTC TTC CCA ATG C -3'
M4:	5'- CCC AAT GCA GGG AGA TCT ATT ACG TAA G -3'
M5:	5'- CGT CGA GAT AGA TCT TTC GAA GAC TGG -3'
M6:	5'- CGA GAT GTC TAC TTT AGA TCT TGG CAA GCA CG -3'
M7:	5'- GGT ATT GAC ATA GAT CTT GGG AAG AAA TAT G -3'
M8:	5'- GGG AAG AAA GAT CTA GAT ATC TGC CCG -3'
M9:	5'- GAT ATC TGC CCA GAT CTT CAT AAT ATG G -3'
M10:	5'- GGC ATC CAG GAT GGG TTA GAT CTA CTG CTC CAG GAC AGC G -3'
M11:	5'- CTG CTC CAG GAA GAT CTG GAG GTA CGA G -3'
M12:	5'- GAG ATT GAG CAA GAT CTC GAC TGT GGA G -3'
M13:	5'- GAA GAG ATC CTA GAT CTG GTG CTG TCT GCC -3'
M14:	5'- CCT GAT CAC GGT AGA TCT TGC CAT GAC AGA GG -3'
M15:	5'- GCT GTC TGC CAT AGA TCT GGA GGC AGC TG -3'
M16:	5'- GCA GCT GTT GCA GAT CTG GCC ATG GC -3'

b) <u>Testing of the eIF-5A mutants for cellular eIF-5A activity in the yeast Saccharomyces cerevisiae</u>

The eIF-5A encoding sequences were cloned between the BamHI and XbaI sites of the yeast shuttle vector pRSGAL using polymerase chain reaction (PCR) technology. In order to test the complementation capacity of the various eIF-5A mutants in an eIF-5A-deficient yeast Saccharomyces cerevisiae, plasmid shuffle technology was employed as described in <u>Mol.Gen.Genet.</u> <u>244</u>, 646-652 (1994). The results are summarized in Table I; several eIF-5A mutants, namely peIF-5AM4 to peIF-5AM10, peIF-5AM13, peIF-5AM14, peIF-5AM16 and peIF-5AM13Δ were identified as non-functional eIF-5A mutant proteins.

c) <u>In vitro binding of non-functional eIF-5A mutant proteins to the HIV-1 Rev trans-activator protein using gel-retardation assays</u>

The wild-type and all non-functional eIF-5A genes were cloned between the BamHI and EcoRI sites of the prokaryotic expression vector pGEX-3X (Pharmacia) using PCR technology, expressed as glutathione-S-transferase (GST) - fusion proteins in Escherichia coli and purified. The various GST-eIF-5A fusion proteins were then subsequently used in gel-retardation assays in combination with ^{32}P-labelled RRE RNA and recombinant Rev protein (Nature 342, 816-819 [1989]) as described in Biochemistry 32, 10497-10505 (1993).

First, the effect of GST-eIF-5A wild-type protein on RRE:Rev complexes was tested. As shown in Figure 1A, addition of GST-eIF-5A to Rev-containing RRE RNA binding reactions resulted in the detection of a higher molecular weight complex with clearly decreased mobility (Fig. 1A, lane 5 vs. 4). This distinct signal was not detectable when RRE RNA was incubated with GST or GST-eIF-5A alone (Fig.1A, lane 2 and 3).

Next it was tested whether the observed eIF-5A-specific signal is dependent on the presence of an activation domain within the Rev *trans*-activator protein. For this, the recently described mutant proteins Rev9Δ14 and Rev11Δ14 (Biochemistry 32, 8945-8954 [1993]) were employed in this assay. Both Rev proteins are characterized by internal C-terminal deletions and display binding affinities and specificities for RRE RNA comparable with wild-type Rev. Importantly, the activation domain is completely removed in Rev9Δ14, while still present in the Rev11Δ14 protein. The lower molecular weight of both proteins resulted in band shifts of the RRE:protein complex which were difficult to resolve in gel retardation assays. Nevertheless, the data clearly demonstrate that the activation domain deletion mutant Rev9Δ14 is incapable of interacting with GST-eIF-5A (Fig. 1A, lane 6 vs. 7). In contrast, addition of GST-eIF-5A to the RRE:Rev11Δ14 binding reaction resulted in a new signal (Fig. 1A, lane 8 vs. 9; slower moving spot in lane 9), indicative of interaction of eIF-5A with the Rev activation domain.

Finally, the binding capacities of the various non-functional eIF-5A mutant proteins were tested in the same assay. As shown in Figure 1B, most of the GST-eIF-5A mutant proteins lost their ability to recognize the RRE:Rev complex (lanes 4 to 10 and lane 13). However, the three non-functional mutant proteins GST-eIF-5AM13, GST-eIF-5AM14 and GST-eIF-5AM13Δ displayed binding behaviours which were comparable with that of

the eIF-5A wild-type protein (Fig.1B, lane 3 vs lanes 11, 12 and 14).

Taken together, the data shows that eIF-5AM13, eIF-5AM14 and eIF-5AM13Δ mutants fulfill the requirements for an inhibitor of HIV-1 Rev function, namely:
(1) loss of endogenous eIF-5A function (see yeast complementation; Table I); and
(2) binding activity to the HIV-1 Rev protein (see Rev in vitro binding; Table I and Fig. 1B).

d) Constitutive inhibition of HIV-1 replication in human T-cells by retroviral mediated gene transfer of trans-dominant eIF-5A mutants

In order to test the HIV-1 inhibitory phenotype of eIF-5AM13, eIF-5AM14 and eIF-5AM13Δ, the respective eIF-5A coding regions were cloned in the Xho I site of the retroviral vector pBC140, in vitro packaged using the Am12 cell line and delivered into human CEM T-cells for constitutive expression as described in Proc. Natl. Acad. Sci. 89, 9870-9874 (1992). Expression of the eIF-5A wild-type gene, the gene encoding eIF-5AM9 and the retroviral vector alone served as controls in these experiments.

HIV-1 challenging experiments were performed, using a TCID of 2000/ml of the HIV-1 SF2 strain. At day 10 after SF2 infection, aliquots of the culture media were collected to assay HIV-1 replication as p24 Gag protein using an antigen capture assay (ELISA). As shown in Figure 2, HIV-1 replication was significantly reduced in each of the three independently cloned CEM cell lines (termed A, B, C) expressing either eIF-5AM13, eIF-5AM14 or eIF-5AM13Δ (Fig. 2C, 2D and respectively 2E; left panels), when compared with CEM cells expressing the eIF-5AM9 mutant protein (CEM-eIF-5AM9; Fig. 2B). Maximal virus replication was detected in CEM cells expressing the parental pBC140 vector (CEM-vector; Fig. 2A) or the eIF-5A wild-type protein (CEM-eIF-5Awt; Fig. 2A). The observed inhibitory effects were not due to a general inhibition of cell proliferation as indicated by comparable cell counts in the experiments (Fig.2; right panels).

In conclusion, these experiments demonstrate that (1) constitutive expression of the eIF-5AM13, eIF-5AM14 or eIF-5AM13Δ proteins is not toxic in human CEM T-cells and (2) that eIF-5AM13, eIF-5AM14 and eIF-5AM13Δ are inhibitors of HIV-1 replication.

HIV is the predominant etiologic agent of AIDS (acquired immunodeficiency syndrome); HTLV-I is causing i.a. ATL (adult T-cell leukemia); HTLV-II is etiologically related to some cases of variant T-cell hairy cell leukemia. The HIV Rev and the HTLV-I Rex trans-activators have been shown to be essential for viral replication in culture. In addition it was demonstrated that HTLV-I Rex is able to functionally substitute for the Rev protein in HIV. Therefore, Rev and Rex are potential targets for therapeutic intervention in afflicted patients. eIF-5A mutants according to the invention act as effective competitive inhibitors of wild-type Rev and/or, as a consequence of Rex's potential to substitute functionally for Rev in HIV, of wild-type Rex function. Therefore, these eIF-5A mutants also act as effective inhibitors of HIV and/or HTLV-I and/or HTLV-II replication. They could thus be used to protect the lymphoid and/or myeloid cells of patients exposed to infection and are thus indicated for the treatment of diseases caused by HTLV-I or HTLV-II or, respectively, of HIV-induced diseases including AIDS and ARS or ARC (AIDS-related syndrome or complex). Further, being able to inhibit both HTLV-I Rex and HIV Rev protein action, they are thus indicated for use in the treatment of HIV-induced diseases. This property may be of particular value in patients coinfected with more than one of these viral pathogens or in those whose infection has not been distinguished between these two agents.

The invention is thus indicated for use in the prophylaxy and therapy of viral, particularly retroviral diseases such as ATL, AIDS , ARS (or ARC), SIV (simian immunodeficiency virus) such as SIV_{mac}, FIV (feline immunodeficiency virus), EIAV (equine infectious anemia virus), visna virus and bovine immunodeficiency virus infections, especially human retroviral diseases, more especially human retroviral diseases caused by pathogens regulated by the rex or rev gene or equivalents thereof, such as ATL, AIDS and ARS (or ARC). Of particular benefit is thereby the multivalent aspect of the repressor effect since it is of advantage in the treatment of multiple, especially double infection by virus, such as is often seen in i.v. drug users coinfected by HIV and HTLV-I, or in treatment in situations of single infection with increased risk of further infection, such as in HIV infection, or in prophylaxy in situations where it is desired to protect against infection by a spectrum of different viral species.

The therapeutic potential of the invention is readily apparent since the repression of e.g. the Rex function of HTLV-I and the Rev function of HIV blocks viral replication, thus lowering the viral burden by preventing the formation of infective virus particles, and is thus expected to perpetuate the latent stage of infection. Thus the cells of subjects already infected with the HIV virus but also having, integrated into their genome, the gene for an eIF-5A mutant repressor would remain functional and the subjects indefinitely free of symptoms of disease, without the need for further long-term therapy.

Viewed in this light the genes according to the invention are pharmaceuticals in themselves, for single or multiple administration either directly in vivo or indirectly in vitro, preferably as part of a vector, e.g. a retroviral or plasmid vector, in a form suitable for achieving delivery in a functional form into target mammalian cells; for example insertion of genes that encode such inhibitors of viral replication may be effected in vitro into cells of patients by direct implantation into the genome of lymphoid and/or myeloid cells derived from infected individuals and these cells may be administered to the donor patient after insertion has been effected. Since HTLV-I and HTLV-II as well as HIV replicate in various types of T-cells, the diseases they cause would appear to be particularly suited. Thus, T-cells are desirable target cells for genetic modification using the genes according to the invention. In addition, monocyte/macrophage cells, which are targets of HIV infection, are also appropriate cells for modification. Preferably hematopoietic stem cells are modified, thereby providing long-lasting protection in progeny cells of multiple hematopoietic lineage.

One application of this would thus parallel the gene therapy concept disclosed in e.g. T. Friedmann, Science 244 (1989) 1275 or P.M. Lehn, Bone Marrow Transplant. 1 (1987) 243: hematopoietic stem cells are extracted from e.g. AIDS/ATL patients and cultivated in vitro, the mutated gene according to the invention, coding for an inhibitor for the function to be repressed, namely the Rev/Rex function, is implanted into these cells using retroviral vectors; the now virus-resistant stem cells are returned to the immune system of the original patient, where they are expected to proliferate in view of their acquired selective advantage over non-treated stem cells; in due time the population of hematopoietic cells will consist entirely of cells producing the repressor factor and be virus-resistant.

Methods on how to effect this are known in the art, see e.g. USP 4'868'116. Vectors, e.g. retroviral or plasmid vectors for delivering the mutated genes according to the invention into target mammalian cells such as bone marrow cells are disclosed or referred to in, e.g., Science 244 (1989) 1275. Thus, for example, various Rev and/or Rex transdominant genes are cloned into retroviral vector systems. After retroviral-mediated gene delivery into e.g. HIV-infected human cell lines the inhibitory effect of the mutants is readily ascertained by inhibition of viral production. The above therapeutic concept is an example of intracellular immunization as envisaged in D. Baltimore, Nature 335 (1988) 395. In brief, the concept involves insertion of a gene that encodes a repressor of some vital function of a selected virus into the particular target cells which that virus infects (e.g., certain T-cells and monocyte/macrophage cells in the case of the virus causing AIDS). Application of this approach to therapy with a repressor of any virus is conditional upon establishment that potential vectors for genes encoding such mutant proteins and possible methods of inserting these vectors into the proper cells, which have been identified in model systems, constitute effective and safe intracellular delivery systems for human or animal applications. The concept can thus be put to the test of experimental verification only with difficulty in view of the ethical barriers presently preventing gene therapy. However, the first such human genetic experiments have now started, with both safety and therapeutic goals [see e.g. G.T. Nabel, Human Gene Therapy 5 (1994) 79-82]. It is to be expected that very shortly after the innocuity of the procedure has been demonstrated these pioneer experiments will be followed by similar trials with therapeutic goals, first in life-threatening conditions such as AIDS disease, and the present invention would appear to be well suited for use in such trials (see e.g. T. Friedmann, Science 244 [1989] 1279, second column, "Infectious diseases").

A further mode of using the invention includes insertion not of a gene but of a repressor protein according to the invention into target cells. Administration e.g. orally or parenterally is effected in conventional manner in a form allowing intracellular penetration, such as by liposome-mediated delivery. For these uses the exact dosage will of course vary depending upon the compound employed, mode of administration and treatment desired; ascertaining the most suitable dosage in a particular situation is within the skill of the man of the art.

It is to be understood that various combinations or changes in form and detail can be made to the invention as described above without departing from the scope of the present invention. It is also to be understood that further mutants as described above, including mutants among the specific mutants already constructed and disclosed herein but which have not been characterized but may be characterized upon more detailed investigation as being inhibitory and/or multivalent, and further mutants in accordance with the principles described above but not specifically disclosed herein, also fall within the scope of the present invention.

Explanation of the Figures:

Figure 1: In vitro RNA gel-shift experiments.

Figure 2: HIV-1 challenge experiments. p24Gag levels are shown at left. Cell counts of the same experiments are shown at right:

A: CEM-vector / eIF-5A wt
B: CEM-eIF-5AM9
C: CEM-eIF-5AM13
D: CEM-eIF-5AM14
E: CEM-eIF-5AM13Δ

Figure 3 (SEQ ID No. 17): cDNA and amino acid coding sequence for wild-type human eIF-5A (J.Biol.Chem. 264 [1989] 1581) (SEQ ID No. 17); the lysine shown at amino acid position 50 is converted posttranslationally to hypusine. The amino acids of wild-type eIF-5A that were mutated as shown in Table 1 are written in italics. The specific oligonucleotides used (Table 2; SEQ ID No. 1 to No. 16) correspond to the following nucleotide positions in Figure 3 (SEQ ID No. 17):

M1: 3-29 (27 nucleotides)
M2: 12-38 (27 nucleotides)
M3: 34-61 (28 nucleotides)
M4: 54-81 (28 nucleotides)
M5: 120-146 (27 nucleotides)
M6: 123-154 (32 nucleotides)
M7: 178-208 (31 nucleotides)
M8: 196-222 (27 nucleotides)
M9: 211-238 (28 nucleotides)
M10: 277-316 (40 nucleotides)
M11: 301-328 (28 nucleotides)
M12: 364-391 (28 nucleotides)
M13: 391-420 (30 nucleotides)
M14: 399-430 (32 nucleotides)
M15: 411-439 (29 nucleotides)
M16: 433-458 (26 nucleotides)
M13Δ: 391-421 (30 nucleotides, with position 409 untranslated, followed by a STOP codon at positions 422-424)

and led to the amino acid changes shown in Table 1 in the sequence for the wild-type protein.

Sequence listing

(1) GENERAL INFORMATION:
(i) APPLICANT:
(A) NAME: Sandoz Ltd.
(B) STREET: Lichtstrasse 35
(C) CITY: Basle
(E) COUNTRY: Switzerland
(F) POSTAL CODE (ZIP): CH-4002
(G) TELEPHONE: 61-324 5269
(H) TELEFAX: 61-322 7532

(A) NAME: Hauber, Joachim
(B) STREET: Nothartgasse 21/10
(C) CITY: Vienna
(D) STATE:
(E) COUNTRY: Austria
(F) POSTAL CODE (ZIP): A-1130

(ii) TITLE OF INVENTION: MUTANT PROTEINS

(iii) NUMBER OF SEQUENCES: 17

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE:

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: WO PCT/EP 96/.....

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: GB 9502771.0
(B) FILING DATE: 13-FEB-1995
(A) APPLICATION NUMBER: GB 9513505.9
(B) FILING DATE: 03-JUL-1995

(2) INFORMATION FOR SEQ ID NO: 1 (M1):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE:
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Synthetic
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: -
(I) FILING DATE: -
(J) PUBLICATION DATE: -
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GGC AGA TGA AGA TCT CTT CGA GAC AGG

(3) INFORMATION FOR SEQ ID NO: 2 (M2):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE:
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Synthetic
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: -
(I) FILING DATE: -
(J) PUBLICATION DATE: -
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CTT GGA CTT CGA AGA TCT AGA TGC AGG

(4) INFORMATION FOR SEQ ID NO: 3 (M3):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE:
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Synthetic
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: -
(I) FILING DATE: -
(J) PUBLICATION DATE: -
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GCA GGG GCC GCA GAT CTC TTC CCA ATG C

(5) INFORMATION FOR SEQ ID NO: 4 (M4):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE:
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Synthetic
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: -
(I) FILING DATE: -
(J) PUBLICATION DATE: -
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CCC AAT GCA GGG AGA TCT ATT ACG TAA G

(6) INFORMATION FOR SEQ ID NO: 5 (M5):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE:
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Synthetic
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: -
(I) FILING DATE: -
(J) PUBLICATION DATE: -
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CGT CGA GAT AGA TCT TTC GAA GAC TGG

(7) INFORMATION FOR SEQ ID NO: 6 (M6):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE:
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: -
(I) FILING DATE: -
(J) PUBLICATION DATE: -
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CGA GAT GTC TAC TTT AGA TCT TGG CAA GCA CG

(8) INFORMATION FOR SEQ ID NO: 7 (M7):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE:
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: -
(I) FILING DATE: -
(J) PUBLICATION DATE: -
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GGT ATT GAC ATA GAT CTT GGG AAG AAA TAT G

(9) INFORMATION FOR SEQ ID NO: 8 (M8):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE:
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: -
(I) FILING DATE: -
(J) PUBLICATION DATE: -
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GGG AAG AAA GAT CTA GAT ATC TGC CCG

(10) INFORMATION FOR SEQ ID NO: 9 (M9):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE:
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: -
(I) FILING DATE: -
(J) PUBLICATION DATE: -
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GAT ATC TGC CCA GAT CTT CAT AAT ATG G

(11) INFORMATION FOR SEQ ID NO: 10 (M10):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE:
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: -
(I) FILING DATE: -
(J) PUBLICATION DATE: -
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGC ATC CAG GAT GGG TTA GAT CTA CTG CTC CAG GAC AGC G

(12) INFORMATION FOR SEQ ID NO: 11 (M11):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE:
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: -
(I) FILING DATE: -
(J) PUBLICATION DATE: -
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CTG CTC CAG GAA GAT CTG GAG GTA CGA G

(13) INFORMATION FOR SEQ ID NO: 12 (M12):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE:
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: -
(I) FILING DATE: -
(J) PUBLICATION DATE: -
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GAG ATT GAG CAA GAT CTC GAC TGT GGA G

(14) INFORMATION FOR SEQ ID NO: 13 (M13):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE:
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: -
(I) FILING DATE: -
(J) PUBLICATION DATE: -
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GAA GAG ATC CTA GAT CTG GTG CTG TCT GCC

(15) INFORMATION FOR SEQ ID NO: 14 (M14):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE:
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: -
(I) FILING DATE: -
(J) PUBLICATION DATE: -
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CCT GAT CAC GGT AGA TCT TGC CAT GAC AGA GG

(16) INFORMATION FOR SEQ ID NO: 15 (M15):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE:
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: -
(I) FILING DATE: -
(J) PUBLICATION DATE: -
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GCT GTC TGC CAT AGA TCT GGA GGC AGC TG

(17) INFORMATION FOR SEQ ID NO: 16 (M16):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE:
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: synthetic
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: -
(I) FILING DATE: -
(J) PUBLICATION DATE: -
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GCA GCT GTT GCA GAT CTG GCC ATG GC

(18) INFORMATION FOR SEQ ID NO: 17 (wt):
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 465 base pairs (and 154 amino acid residues)
(B) TYPE: cDNA
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: J.Biol.Chem. 264 (1989) 1578-1583
(I) FILING DATE: -
(J) PUBLICATION DATE: 1989
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
           10                                  30                                  50
ATG GCA GAT GAC TTG GAC TTC GAG ACA GGA GAT GCA GGG GCC TCA GCC ACC
Met Ala Asp Asp Leu Asp Phe Glu Thr Gly Asp Ala Gly Ala Ser Ala Thr
  1                                              10

                        70                                  90
TTC CCA ATG CAG TGC TCA GCA TTA CGT AAG AAT GGC TTT GTG GTG CTC AAA
Phe Pro Met Gln Cys Ser Ala Leu Arg Lys Asn Gly Phe Val Val Leu Lys
        20

      110                                 130                                 150
GGC CGG CCA TGT AAG ATC GTC GAG ATG TCT ACT TCG AAG ACT GGC AAG CAC
Gly Arg Pro Cys Lys Ile Val Glu Met Ser Thr Ser Lys Thr Gly Lys His
                    40                                  50

                  170                                 190
GGC CAC GCC AAG GTC CAT CTG GTT GGT ATT GAC ATC TTT ACT GGG AAG AAA
Gly His Ala Lys Val His Leu Val Gly Ile Asp Ile Phe Thr Gly Lys Lys
                                60

    210                                 230                                 250
TAT GAA GAT ATC TGC CCG TCA ACT CAT AAT ATG GAT GTC CCC AAC ATC AAA
Tyr Glu Asp Ile Cys Pro Ser Thr His Asn Met Asp Val Pro Asn Ile Lys
    70                                  80

                270                                 290
AGG AAT GAC TTC CAG CTG ATT GGC ATC CAG GAT GGG TAC CTA TCA CTG CTC
Arg Asn Asp Phe Gln Leu Ile Gly Ile Gln Asp Gly Tyr Leu Ser Leu Leu
                90                                  100

  310                               330                                 350
CAG GAC AGC GGG GAG GTA CGA GAG GAC CTT CGT CTC CCT GAG GGA GAC CTT
Gln Asp Ser Gly Glu Val Arg Glu Asp Leu Arg Leu Pro Glu Gly Asp Leu
                                110

              370                                 390                                 4
GGC AAG GAG ATT GAG CAG AAG TAC GAC TGT GGA GAA GAG ATC CTG ATC ACG
Gly Lys Glu Ile Glu Gln Lys Tyr Asp Cys Gly Glu Glu Ile Leu Ile Thr
120                                 130

10                                430                                 450
GTG CTG TCT GCC ATG ACA GAG GAG GCA GCT GTT GCA ATC AAG GCC ATG GCA
Val Leu Ser Ala Met Thr Glu Glu Ala Ala Val Ala Ile Lys Ala Met Ala
              140                                 150

AAA TAA
Lys End
```

Claims:

1. An eIF-5A mutant protein which inhibits Rev function and thus possesses an inhibitory phenotype with respect to Rev function, or a gene or a DNA or cDNA sequence coding therefor.

2. A protein as defined in claim 1 which is devoid of substantial endogenous eIF-5A function, or a gene or a DNA or cDNA sequence coding therefor.

3. A protein as defined in claim 1 or 2 which is the eIF-5AM13, eIF-5AM14 or eIF-5aM13Δ protein, or a gene or a DNA or cDNA sequence coding therefor.

4. A prokaryotic or eukaryotic host cell transformed or transfected with a gene or a DNA or cDNA sequence as defined in claim 1 or 2 in a manner allowing the host cell to express the gene or the DNA or cDNA sequence.

5. A biologically functional plasmid or viral DNA vector including a gene or a DNA or cDNA sequence as defined in claim 1 or 2.

6. A prokaryotic or eukaryotic host cell stably transformed or transfected with a biologically functional plasmid or viral DNA vector including a gene or a DNA or cDNA sequence as defined in claim 1 or 2.

7. A process for the preparation of a gene or a DNA or cDNA sequence as defined in claim 1 or 2 which comprises isolating the corresponding wild-type gene from an appropriate expression system, inserting this gene into an appropriate cloning system, introducing the desired mutation into the gene and recovering the resultant mutant from the clones having the desired mutation.

8. A process for the production of a protein as defined in claim 1 or 2 which comprises culturing under suitable nutrient conditions a prokaryotic or eukaryotic host cell transformed or transfected with a gene or a DNA or cDNA sequence as defined in claim 1 or 2 in a manner allowing the host cell to express the protein, and optionally isolating the desired polypeptide product of the expression.

9. A pharmaceutical composition comprising a protein as defined in claim 1 or 2, together with at least one pharmaceutically acceptable carrier, adjuvant or diluent, or in the form of cells taken from a patient's body and treated in vitro prior to reinsertion.

10. Use of a protein as defined in claim 1 or 2 or of a gene or a DNA or cDNA sequence coding therefor in the treatment of diseases caused by retroviruses dependent on eIF-5A for Rev function.

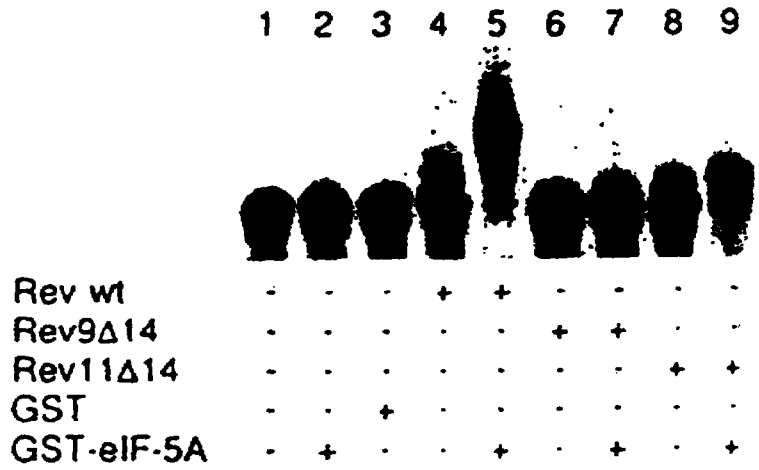
11. Use of a protein as defined in claim 1 or 2 or of a gene or a DNA or cDNA sequence coding therefor in the treatment of diseases caused by HIV, HTLV-I and/or HTLV-II.

12. A protein as defined in claim 1 or 2 or a gene or a DNA or cDNA sequence coding therefor, for use as a pharmaceutical.

13. Use of a protein as defined in claim 1 or 2 in the preparation of a medicament for the treatment of diseases caused by retroviruses dependent on eIF-5A for Rev function.

Fig. 1

A


1 2 3 4 5 6 7 8 9
Rev wt - - - + + - - - -
Rev9Δ14 - - - - - + + - -
Rev11Δ14 - - - - - - - + +
GST - - + - - - - - -
GST-eIF-5A - + - - + - + - +

B


1 2 3 4 5 6 7 8 9 10 11 12 13 14
free probe
no eIF-5A
wt
M4
M5
M6
M7
M8
M9
M10
M13
M14
M16
M13Δ

2/3

Fig.2

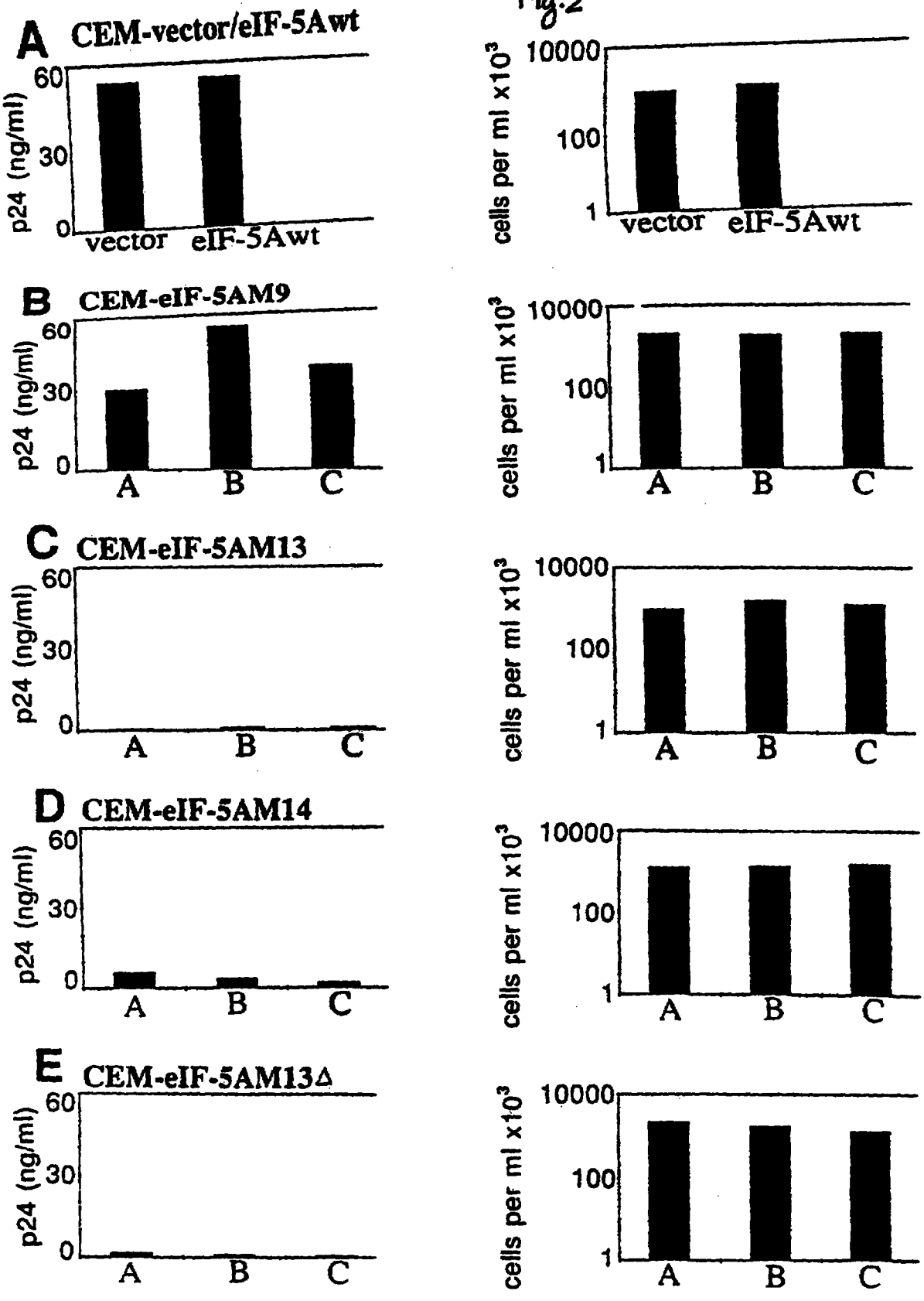

A CEM-vector/eIF-5Awt
p24 (ng/ml)
60
30
0
vector
eIF-5Awt
cells per ml x10³
10000
100
1
vector
eIF-5Awt
B CEM-eIF-5AM9
p24 (ng/ml)
60
30
0
A
B
C
cells per ml x10³
10000
100
1
A
B
C
C CEM-eIF-5AM13
p24 (ng/ml)
60
30
0
A
B
C
cells per ml x10³
10000
100
1
A
B
C
D CEM-eIF-5AM14
p24 (ng/ml)
60
30
0
A
B
C
cells per ml x10³
10000
100
1
A
B
C
E CEM-eIF-5AM13Δ
p24 (ng/ml)
60
30
0
A
B
C
cells per ml x10³
10000
100
1
A
B
C

Figure 3

```
           10                                  30                                  50
ATG GCA GAT GAC TTG GAC TTC GAG ACA GGA GAT GCA GGG GCC TCA GCC ACC
Met Ala Asp Asp Leu Asp Phe Glu Thr Gly Asp Ala Gly Ala Ser Ala Thr
1                                   10

                        70                                  90
TTC CCA ATG CAG TGC TCA GCA TTA CGT AAG AAT GGC TTT GTG GTG CTC AAA
Phe Pro Met Gln Cys Ser Ala Leu Arg Lys Asn Gly Phe Val Val Leu Lys
        20

       110                                 130                                 150
GGC CGG CCA TGT AAG ATC GTC GAG ATG TCT ACT TCG AAG ACT GGC AAG CAC
Gly Arg Pro Cys Lys Ile Val Glu Met Ser Thr Ser Lys Thr Gly Lys His
                    40                                  50

                    170                                 190
GGC CAC GCC AAG GTC CAT CTG GTT GGT ATT GAC ATC TTT ACT GGG AAG AAA
Gly His Ala Lys Val His Leu Val Gly Ile Asp Ile Phe Thr Gly Lys Lys
                                60

    210                                 230                                 250
TAT GAA GAT ATC TGC CCG TCA ACT CAT AAT ATG GAT GTC CCC AAC ATC AAA
Tyr Glu Asp Ile Cys Pro Ser Thr His Asn Met Asp Val Pro Asn Ile Lys
    70                                  80

                270                                 290
AGG AAT GAC TTC CAG CTG ATT GGC ATC CAG GAT GGG TAC CTA TCA CTG CTC
Arg Asn Asp Phe Gln Leu Ile Gly Ile Gln Asp Gly Tyr Leu Ser Leu Leu
                90                                  100

  310                               330                                 350
CAG GAC AGC GGG GAG GTA CGA GAG GAC CTT CGT CTC CCT GAG GGA GAC CTT
Gln Asp Ser Gly Glu Val Arg Glu Asp Leu Arg Leu Pro Glu Gly Asp Leu
                                110

                370                                 390                                 4
GGC AAG GAG ATT GAG CAG AAG TAC GAC TGT GGA GAA GAG ATC CTG ATC ACG
Gly Lys Glu Ile Glu Gln Lys Tyr Asp Cys Gly Glu Glu Ile Leu Ile Thr
120                                 130

10                              430                                 450
GTG CTG TCT GCC ATG ACA GAG GAG GCA GCT GTT GCA ATC AAG GCC ATG GCA
Val Leu Ser Ala Met Thr Glu Glu Ala Ala Val Ala Ile Lys Ala Met Ala
                140                                 150

AAA TAA
Lys End
```