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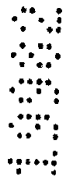
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Abstract of the disclosure**Nucleic acid constructs containing genes encoding transport signals**

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Nucleic acid constructs are disclosed which possess a nuclear retention signal which is linked, downstream in the reading direction, to a transgene. The nuclear retention signal can regulate the presence of the transcription product in the cell nucleus or else the intracellular transport of the transcription product.

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AUSTRALIA

Patents Act 1990

**ORIGINAL
COMPLETE SPECIFICATION
STANDARD PATENT**



Application Number:

Lodged:



Invention Title:

NUCLEIC ACID CONSTRUCTS CONTAINING GENES ENCODING TRANSPORT
SIGNALS



The following statement is a full description of this invention, including the
best method of performing it known to us :-

Description

5 Nucleic acid constructs containing genes encoding transport signals

The present application relates to nucleic acid constructs which can be used in genetic manipulation and, in particular, in the prophylaxis or therapy of diseases (termed gene therapy below).

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In gene therapy, genes are introduced into the organism within the intention of expressing them in the organism. The regulation of the expression of these genes is of significance for the prophylactic or therapeutic effect of gene therapy.

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Patent Applications PCT/GB95/02000, PCT/EP 95/03370, PCT/EP 95/03371, PCT/EP 95/03368, PCT/EP 95/03339 describe gene expression regulators. These regulators comprise an activator sequence whose function is, for example, the cell-specific or virus-specific activation of basal transcription. The DNA sequence of this activator sequence is linked, by its 3' end, to the 5' end of a promoter module. The structural gene is in turn linked, by its 5' end, to the 3' end of the promoter module.

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The promoter module comprises nucleic acid sequences for binding the transcription factors of the CDF and CHF or E2F and CHF families. In the G0 and G1 phases of the cell cycle, this binding leads to inhibition of the upstream activator sequence and consequently to inhibition of the transcription of the structural gene which is located downstream (i.e. in the direction of transcription).

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In the G0 and G1 phases of cell division, the DNA contained in the cell is in the diploid state. The cell is at rest in the G0 phase and is inhibited in its cell-cycle progression in the G1 phase. The G1 phase is followed by the S phase, in which DNA synthesis takes place and in which the genome is replicated. There then follows the G2 phase, in which the cell is in the tetraploid state. The G2 phase is followed by cell division (mitosis = M phase). The daughter cells pass into the G0 or G1 states.

Consequently, combining a cell-specific or virus-specific activator sequence with a promoter module which inhibits this activator sequence in the G0 and G1 phases makes it possible to regulate the expression of a structural gene both in a cell-specific or virus-specific manner and in a cell-cycle-specific manner (i.e. restricted to the S and G2 phases).

The combination of an activator sequence and a promoter module is termed a chimeric promoter. While there are numerous possibilities for applying chimeric promoters in gene therapy, the shortcomings of these promoters also impose a number of limitations.

Examples of these limitations are

- a weak activator sequence, which effects too low a transcription of the structural gene,
- the use of an activator sequence which cannot be adequately inhibited in a cell-cycle-dependent manner by the chosen promoter module,
- the restriction to two (for example cell or virus-specific and cell-cycle-specific) regulators of the transcription of the structural gene, and
- inadequate persistence of the transcription product in the cell nucleus
- or inadequate intracellular transport of the transcription product of the structural gene which has been introduced into the cell.

It is an object of the present invention to make available nucleic acid constructs which make it possible to regulate the expression of foreign genes (transgenes) in the host cells in a precise manner. The present invention relates, therefore, to nucleic acid constructs which comprise at least the following components, in the reading direction from the 5' end to the 3' end:



- a) a first nonspecific, cell-specific, virus-specific, metabolically and/or cell-cycle-specifically activatable promoter sequence or enhancer sequence (I) which activates the basal transcription of a transgene,
- b) a transgene which, as structural gene, encodes an active compound, and
- c) a nuclear retention signal whose cDNA is linked, directly or indirectly, at the 5' end, to the 3' end of the structural gene (b).
- d) an additional promoter sequence or enhancer sequence (II) which activates the basal transcription of a nuclear export factor, and
- e) a nucleic acid which encodes a nuclear export factor which binds to the transcription product of the nuclear retention signal (c) and thereby mediates transport of the transcription product of the transgene out of the cell nucleus.

Preferably, the transcription product of the nuclear retention signal has a structure which binds a nuclear export factor.

The first (I) promoter sequence or enhancer sequence (a) and the second (II) promoter sequence or enhancer sequence (d) can be identical or different and at least one of the components a) and d) can be nonspecifically, cell-specifically, virus-specifically, metabolically, in particular by hypoxia, or cell-cycle-specifically activatable.

Within the scope of the present invention, at least one of the promoter sequences or enhancer sequences (a) and (d) can be a chimeric promoter in which the promoter



module CDE-CHR or E2FBS-CHR interacts with an adjacent upstream cell-specifically, virus-specifically or metabolically activatable activator sequence and can thereby influence, in particular inhibit, the expression of a downstream gene.

5 The components a) and d) can be an activator-responsive promoter unit. Constructs of this nature additionally possess the following components:

f) at least one promoter sequence or enhancer sequence which is nonspecifically, virus-specifically, metabolically or cell-specifically and/or cell-cycle-specifically activatable,

g) at least one activator subunit which is located downstream of the promoter sequence or enhancer sequence (f) and is activated, in its basal transcription, by the promoter sequence or enhancer sequence (f), and

h) an activator-responsive promoter which is activated by the expression products of one or more activator subunit(s) (g).

In one embodiment of the invention, the nucleic acid constructs are nucleic acid constructs in which the promoter sequence or enhancer sequence (a) and/or (d) and/or the activator-responsive promoter is a chimeric promoter and the activator subunit (g) is a gene for at least one transcription factor which activates the chimeric promoter of the activator-responsive promoter (h).

25 The LexA operator in combination with the SV40 promoter is an example of an activator-responsive promoter (h) which is activated by two activator subunits (g, g'). The activator subunit (g) comprises the cDNA for the LexA DNA-binding protein encoding amino acids 1-81 or 1-202, whose 3' end is linked to the 5' end of the cDNA for the Gal80 protein (amino acids 1-435). The second activator subunit (g')

30 comprises the cDNA of the Gal80-binding domain of the Gal4 protein encoding amino acids 851-881, whose 3' end is linked to the 5' end of the cDNA of the SV40 large T antigen encoding amino acids 126-132, whose 3' end is linked to the 5' end of the cDNA for the HSV-1 VP16 transactivating domain encoding amino acids 406-

488.

The binding sequence for the Gal4 protein in combination with the SV40 promoter is another example of an activator-responsive promoter which is activated by two
5 activator subunits (g, g').

The activating unit (g) comprises the cDNA for the DNA-binding domain of the Gal4 protein (amino acids 1-147), whose 3' end is linked to the 5' end of the cDNA for the Gal80 protein (amino acids 1-435).

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The second activating subunit (g') comprises the cDNA for the Gal80- binding domain of Gal4 (amino acids 851 to 881), whose 3' end is linked to the 5' end of the cDNA of the nuclear localization signal of SV40 (SV40 large T, amino acids 126 to 132) whose 3' end is linked to the 5' end of the cDNA for the HSV-1 VP16
15 transactivating domain encoding amino acids 406-488.

Another example of two activator subunits (g, g') which activate the activator-responsive promoter which is composed of the binding sequence for the Gal4 protein and the SV40 promoter is represented by

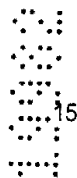
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- the activating unit (g) comprising the cDNA for the cytoplasmic domain of the CD4 T cell antigen (amino acids 397-435), whose 5' end is linked to the 3' end of the cDNA for the HSV-1 VP16 transactivating domain (amino acids 406-488), whose 5' end is in turn linked to the 3' end of the cDNA of the nuclear localization signal of SV40 (SV40 large T, amino acids 16 to 132),
25 and
- the activating unit (g') comprising the cDNA of the nuclear localization signal of SV40 (SV40 large T, amino acids 126 to 132); cDNA for the DNA-binding domain of the Gal4 protein (amino acids 1-147), whose 3' end is linked to the 5' end of the cDNA for the CD4-binding sequence of the p56 lck protein
30 (amino acids 1-71).

Preferably, the gene encoding the nuclear retention signal is selected from the

group consisting of the rev-responsive element (RRE) of HIV-1 or HIV-2, the RRE-equivalent retention signal of retroviruses or the RRE-equivalent retention signal of HBV.

- 5 The nuclear export factor (e) is preferably a gene selected from the group consisting of the rev gene of the viruses HIV-1, HIV-2, maedi-visna virus, caprine arthritis encephalitis virus, equine infectious anemia virus, feline immunodeficiency virus, of retroviruses, of HTLV or the gene of the hnRNP-A1 protein or the gene of the transcription factor TFIII-A. As a rule, the nucleic acid is DNA. The novel nucleic acid constructs are customarily employed as vectors, in particular plasmid vectors or viral vectors.
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As a rule, the transgenes are structural genes which encode a pharmacologically active active compound which is selected from the group consisting of cytokines, growth factors, antibodies or antibody fragments, fusion proteins between ligands, such as antibodies or antibody fragments, and cytokines or growth factors, receptors for cytokines or growth factors, proteins having an antiproliferative or cytostatic effect, angiogenesis inhibitors, coagulation factors, thrombosis-inducing substances and coagulation inhibitors, substances having a fibrinolytic effect, complement-activating proteins, virus coat proteins, bacterial antigens and parasitic antigens, proteins having an effect on the blood circulation, and ribozymes.

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- Preferably, the transgene is a structural gene which encodes a ribozyme which inactivates the mRNA which encodes a protein selected from the group consisting of cell-cycle control proteins, in particular cyclin A, cyclin B, cyclin D1, cyclin E, E2F1-5, cdc2, cdc25C or DP1, or virus proteins or cytokines or growth factors or their receptors.
- 25

- In a particular embodiment, the transgene can be a structural gene which encodes a protein which triggers controlled cell death (apoptosis), in particular sphingomyelinase.
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In another embodiment, the transgene (b) can be a structural gene which encodes

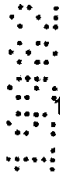
an enzyme which cleaves a precursor of a drug, thereby forming a drug. In a further embodiment, the structural gene can encode a ligand-enzyme fusion protein, with the enzyme cleaving a precursor of a drug, thereby forming a drug, and the ligand binding to a cell surface, preferably to endothelial cells or tumor cells.

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The promoter sequence, enhancer sequence or activator sequence can be selected from the group consisting of gene-regulatory nucleotide sequences which are activated in endothelial cells, smooth muscle cells, striated muscle cells, macrophages, lymphocytes, tumor cells, liver cells, leukemia cells and glia cells, or of promoter sequences of the HBV, HCV, HSV, HPV, EBV, HTLV or HIV viruses.

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The invention also relates to isolated cells or cell lines which harbor a novel nucleic acid construct.



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Cells of this nature can be used for preparing a medicine for the treatment of a disease, with the preparation of the medicine comprising the introduction of the nucleic acid construct into a target cell. Diseases of this nature are frequently accompanied by excessive cell multiplication.



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In order to prepare the medicine, a nucleic acid construct must first of all be introduced into the target cell.



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The novel nucleic acid constructs make it possible to use any promoters, enhancers or activator sequences and, in particular, to augment the persistence in the cell nucleus of the transcription product of the structural gene which has been introduced into the cell or to increase the intracellular transport of the transcription product of the structural gene which has been introduced into the cell.

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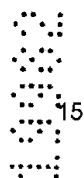
The novel nucleic acid construct for an augmented persistence of the transcription product in the cell nucleus comprises the following components:

- a) a promoter sequence or enhancer sequence I which activates the basal transcription of the structural gene,

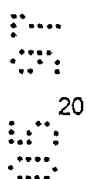
- b) a transgene (structural gene), which is preferably in the form of a cDNA which encodes the desired active compound, and
- c) a nuclear retention signal (NRS), whose cDNA is preferably directly linked, at the 5' end, to the 3' end of the structural gene.

The arrangement of the individual components is depicted, by way of example, in Figure 1.

- 10 The novel nucleic acid construct for increased intracellular transport of the transcription product possesses, in addition to a promoter sequence or enhancer sequence I, which activates the basal transcription of the structural gene, and a transgene, which encodes the desired active compound, a nuclear retention signal (NRS) whose cDNA is linked, at the 5' end, to the 3' end of the structural gene and whose messenger RNA is the structure for binding a nuclear export factor (NEF).



In addition to this, nucleic acid constructs of this nature possess a further promoter sequence or enhancer sequence (II), which activates the basal transcription of the cDNA of the nuclear export factor (NEF) whose expression product binds to the nuclear retention signal (NRS) and thereby mediates transport of the transcription product of the structural gene out of the cell nucleus.



The arrangement of the individual components is depicted, by way of example, in Figure 2.

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In the novel nucleic acid constructs, the promoter sequences or enhancer sequences of components a) or d) can be identical or different and, furthermore, components d) and e) can be located upstream or downstream of components a), b) and c).

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At least one strong promoter sequence or enhancer sequence, for example from CMV (EP-A-0173177) or SV40, or any other promoter sequence or enhancer sequence known to the skilled person, is preferably used as a promoter sequence

or enhancer sequence.

In a preferred embodiment, at least one promoter sequence or enhancer sequence in the novel nucleic acid constructs is cell-specifically, metabolically (for example by hypoxia), virus-specifically or cell-cycle-specifically activatable.

Particular preference is given to

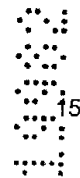
- those promoter sequences or enhancer sequences which activate transcription in endothelial cells, smooth muscle cells, striated muscle cells, hematopoietic cells, lymphocytes, macrophages, glia cells or tumor cells in a cell-specific manner and/or
- promoter sequences or enhancer sequences of the HBV, HCV, HSV, HPV, CMV, EBV, HTLV or HIV viruses and/or
- promoter sequences or enhancer sequences which are metabolically activatable, such as the hypoxia-inducible enhancer (Semenza et al., PNAS 88, 5680 (1991)) or promoter (McBurney et al., Nucleic Acids Res. 19, 5755 (1991); WO 95/21927) and/or
- promoters which are cell-cycle-specifically activatable, such as the promoter of the cdc25C gene, the cyclin A gene, the cdc2 gene (Lucibello et al., EMBO J. 14, 132 (1995), Zwicker et al., EMBO J. 14, 4514 (1995), Zwicker et al., Nucl. Acids Res. 23, 2833 (1995)), the B-myc gene (Lam et al., EMBO J. 12, 2705 (1993)), the DHFR gene (Means et al., Mol. Cell Biol. 12, 1054 (1992)) and the E2F-1 gene (Johnson et al., Genes Dev. 8, 1514 (1994), Hsiao et al., Genes Dev. 8, 15256 (1994)).

In another preferred embodiment, at least one promoter sequence or enhancer sequence in the novel nucleic acid constructs is a chimeric promoter. Within the meaning of this invention, a chimeric promoter constitutes the combination of an upstream, cell-specifically, metabolically or virus-specifically activatable, activator

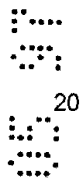
sequence and a downstream promoter module. The promoter module is characterized by a nucleotide sequence which binds the transcription factors of the CDF and CHF or E2F and CHF families and can thereby inhibit activation of the upstream activator sequence in the G0 and G1 phases of the cell cycle (Lucibello et al., EMBO J. 14, 132 (1994), PCT/GB95/02000).

In another preferred embodiment, at least one promoter sequence or enhancer sequence (component a) or d)) in the novel nucleic acid constructs is an activator-responsive promoter unit.

An activator-responsive promoter unit is composed, for its part, of the following components:



f) one or more identical or different promoter sequences or enhancer sequences which is/are, for example, cell-cycle-specifically, metabolically, cell-specifically or virus-specifically activatable or both cell-cycle-specifically and metabolically, cell-specifically or virus-specifically activatable (so-called chimeric promoters),



g) one or more identical or different activator subunits which is/are in each case located downstream of the promoter sequences or enhancer sequences and activated in their basal transcription by these sequences, and



h) an activator-responsive promoter which is activated by the expression products of one or more activator subunits.

The arrangement of the individual components of a preferred activator-responsive promoter unit is depicted by Figure 3.

The insertion of a preferred activator-responsive promoter unit into a novel nucleic acid construct is depicted, for example, by Figure 4.

In their simplest form, activator-responsive promoter units can, for example,

represent chimeric promoter constructs as shown in the diagram in Figure 5.

In another embodiment, novel activator-responsive promoter units can constitute sequences for binding chimeric transcription factors composed of DNA-binding domains, protein-protein interaction domains and transactivating domains.

Preferred structural genes for a pharmacologically active compound are genes for ribozymes, ribozymes having a combined antisense RNA, proteins and glycoproteins which are selected from the group consisting of cytokines, growth factors, receptors for cytokines or growth factors, fusion proteins composed of ligands (e.g. antibodies or antibody fragments) and cytokines or growth factors, proteins having an antiproliferative or cytostatic effect, angiogenesis inhibitors, thrombosis-inducing proteins, blood coagulation inhibitors, complement-activating proteins, coat substances of viruses and coat substances of bacteria.

Particular preference is given to genes for ribozymes which specifically cleave the mRNA of those genes which encode proteins which are particularly involved in the control of the cell cycle.

These proteins include, in particular, cyclin A, cyclin D1, cyclin E, cyclin B, cdc2, E2F1-5, DP1 and cdc25C (La Thangue, *Current Opin. Cell Biol.* 6, 443 (1994); Müller, *Trends Genet.* 11, 173 (1995); Zwicker et al., *EMBO J.* 14, 4514 (1995)).

In a preferred embodiment, the nuclear retention signal (NRS) is a nucleotide sequence which, while it impedes transport through the nuclear membrane of a premessenger RNA which is linked to it, is, on the other hand, a structure for binding an export protein. This export protein mediates transport of the premessenger or messenger RNA which contains an NRS out of the cell nucleus into the cytoplasm. A premessenger or messenger RNA which contains the NRS is consequently secreted from the cell nucleus as a result of binding to the export protein (Fischer et al., *Cell* 82, 475 (1995)).

The NRS is preferably the rev-responsive element (RRE) sequence of retroviruses.

In HIV-1, this RRE is a sequence encompassing 243 nucleotides (nucleotides 7362-7595; Muesing et al., *Nature* 313, 450 (1985)) in the env gene (Malim et al., *Nature* 338, 254 (1989); Kjems et al., *PNAS* 88, 683 (1991)). However, within the meaning of the invention, the nuclear retention signal (NRS) can also be a nucleotide sequence which is homologous and/or functionally similar (analogous), such as the RRE-equivalent of HBV virus (Huang et al., *Mol Cell Biol.* 13, 7476 (1993)).

In the novel nucleic acid constructs, the nuclear export factor (NEF) is a nucleotide sequence which encodes a protein which binds to the mRNA of the NRS and mediates transport of the premessenger RNA or messenger RNA containing an NRS out of the cell nucleus into the cytoplasm (or out of the cytoplasm into the cell nucleus). Within the meaning of the invention, use is made, in particular, of the rev gene from retroviruses, especially from HIV-1 or HIV-2 virus (Daly et al., *Nature* 342, 816 (1989); Emerman et al., *Cell* 57, 1155 (1989); Felber et al., *PNAS* 86, 1495 (1989); Fischer et al., *EMBO J.* 13, 4105 (1994)).

The rev protein of the rev gene of retroviruses binds to the RRE in the pre-mRNA (Iwai et al., *Nucl. Acids Res.* 20, 6465 (1992)) by means of its N-terminal domain (Zapp et al., *Nature* 342, 714 (1989); Malim et al., *Cell* 65, 241 (1991)). The bond between the RRE and the rev protein enables "non-spliced" premessenger RNA, and any other RNA which contains an RRE, to be transported from the cell nucleus into the cytoplasm (Fischer et al., *EMBO J.* 13, 4105 (1994); Fischer et al., *Cell* 82, 475 (1995)) and consequently considerably augments translation.

Within the meaning of the invention, nucleotide sequences which encode proteins which are homologous with, or functionally similar to, the rev protein of HIV-1 (Bogerd et al., *Cell* 82, 485 (1995)), such as the rev gene of visna-maedi virus (VMV; Tiley et al., *J. Virol.* 65, 3877 (1991)) or the rev gene of caprine arthritis encephalitis virus (CAEV; Tiley et al., *J. Virol.* 65, 3877 (1991)), can also be used as NEF's.

Within the meaning of the invention, those genes can also be employed which

encode proteins which, while only possessing low or no homology with the rev protein, are nevertheless functionally similar to the rev protein of HIV-1.

These genes include, for example, the rex gene of HTLV-1 (Cullen, *Microbiol. Rev.* 56, 375 (1992)), and the rev gene of equine infectious anemia virus (EIAV) and of feline immunodeficiency virus (FIV) (Manusco et al., *J. Virol.* 68, 1988 (1994)).

In an alternative embodiment, the NEF's can also be nucleotide sequences for proteins which bring about a secretion of RNA from the nucleus even without this RNA being retained in the nucleus by means of an NRS. These proteins include, for example, the transcription factor TFIIA (Gaddat et al., *Cell* 60, 619 (1990)) or the heterogeneous nuclear ribonucleo- protein A1 (hnRNPA1 protein; Pinol-Roma et al., *Nature* 355, 730 (1992)).

In addition, the nuclear transport proteins include, in a widened sense, heat shock protein 70 (hsc70; Mandell et al., *J. Cell Biol.* 111, 1775 (1990)) and the protein kinase inhibitor CPKI (Fantozzi et al., *J. Biol. Chem.* 269, 2676 (1994), Wen et al., *J. Biol. Chem.* 269, 32214 (1994)).

Features possessed in common by the NEF and its homologous and analogous proteins are a more aminoterminaly located domain for binding the monomeric protein to the RNA of the NRS (*J. Virol* 64, 881 (1990); Kjems et al., *EMBO J.* 11, 1119 (1992)) and a mainly leucine-rich domain (hnRNPA1 is an exception to this) which is required for the transport function of the NEF (Wen et al., *Cell* 82, 463 (1995); Fischer et al., *Cell* 82, 475 (1995); Malim et al., *J. Virol.* 65, 4248 (1991); Venkatesh et al., *Virol.* 178, 327 (1990)).

The nucleic acid constructs are preferably composed of DNA. The term nucleic acid constructs is understood to mean artificial nucleic acid structures which can be transcribed in the target cells. They are preferably inserted into a vector, with plasmid vectors or viral vectors being particularly preferred.

Using the novel nucleic acid constructs, a transgene (component b) can be

expressed both cell-specifically or virus-specifically or under particular metabolic conditions and also cell-cycle-specifically, with the structural gene preferably being a gene which encodes a pharmacologically active compound or else an enzyme which cleaves an inactive precursor of a drug, thereby forming an active drug. The structural gene can be chosen such that this enzyme is expressed as a fusion protein with a ligand and this ligand binds to the surface of cells, for example proliferating endothelial cells or tumor cells.

The present invention also relates to cells of yeasts or mammals which harbor a novel nucleic acid construct. In a particularly preferred embodiment, the nucleic acid constructs are introduced into cell lines which can then be used, after transfection, for expressing the transgene. These cells can consequently be used for preparing a medicine for patients and also in the patient and consequently for treating a disease. A preferred use of the novel nucleic acid construct consists in treating a disease, with the preparation of the medicine comprising the insertion of a nucleic acid construct into a target cell and its virus-specific or target-cell-specific and cell-cycle-specific expression. The disease is frequently a disease which is accompanied by excessive cell multiplication, with the preparation of the medicine comprising the insertion of a nucleic acid construct into a target cell and its virus-specific or target-cell-specific expression in the stage of cell proliferation.

The novel nucleic acid constructs do not occur in nature in this form, i.e. the transgene or structural gene for the active compound or for an enzyme or for a ligand-enzyme fusion protein is not naturally combined with the nuclear retention signal (NRS) and the two are not naturally linked to the promoter I, and this combination, in turn, is not naturally combined with the nucleotide sequence comprising the promoter II and the nuclear export factor (NEF).

The promoters I and II, and the structural gene for the active compound (or for the enzyme), of the novel nucleic acid constructs are chosen in dependence on the intended application.

Some preferred application possibilities are presented below:

1.1 Combination of a strong, non-specific promoter or enhancer with a chimeric promoter for expressing a structural gene

Examples of such combinations are depicted in Figure 6.

1.2 Combination of two different or identical chimeric promoters for expressing a structural gene

Examples of such combinations are depicted in Figure 7.

1.3 Combination of two different or identical promoters or enhancers for expressing a structural gene

Examples of such combinations are depicted in Figure 8.

1.4 Activator-responsive promoter

Examples of such combinations are depicted in Figure 9.

1.5 Multiple combination of different promoters for the multiply controlled expression of a structural gene

Examples of such combinations are depicted in Figure 10.

Depending on the planned use of the nucleic acid constructs, the following embodiments can be selected:

2. Therapy of tumors and chronic inflammations by inhibiting the proliferating endothelium

2.1.a) Selection of the promoters or activator sequences which are activated in endothelial cells

Within the meaning of this invention, the preferred promoters or activator sequences composed of promoters or enhancers include those gene-regulatory sequences or elements for genes which encode proteins which can be detected, in particular, in endothelial cells (or else in cells in the immediate vicinity of proliferating endothelial cells).

Some of these proteins have been described by Borrows et al. (Pharmac. Ther. 64, 155 (1994)) and Plate et al. (Brain Pathol. 4, 207 (1994)). Examples of these endothelial-cell-specific proteins are, in particular:

- brain-specific, endothelial glucose-1-transporter

The promoter sequence was described by Murakami et al. (J. Biol. Chem. 267, 9300 (1992)).

- endoglin

The promoter sequence was described by Bellon et al. (Eur. J., Immunol. 23, 2340 (1993)) and Ge et al. (Gene 138, 201 (1994)).

- VEGF-receptors

Two receptors are distinguished (Plate et al., Int. J. Cancer 59, 520 (1994)):

- VEGF receptor 1 (flt-1)
(de Vries et al., Science 255, 989 (1992)) and the
- VEGF receptor 2 (flk-1, KDR)
(Terman et al., BBRC 187, 1579 (1992)).

Both receptors are almost exclusively found on endothelial cells (Senger et al., Cancer Metast. Rev. 12, 303 (1993)).

- other endothelial-cell-specific receptor tyrosine kinases

- til-1 or til-2
(Partanen et al., Mol. Cell. Biol. 12, 1698 (1992), Schnürch and Risau, Development 119, 957 (1993), Dumont et al., Oncogene 7, 1471 (1992))
- B61 receptor (Eck receptor)
(Bartley et al., Nature 368, 558 (1994), Pandey et al., Science 268, 567 (1995), van der Geer et al., Ann. Rev. Cell. Biol. 10, 251 (1994))

- B61

The B61 molecule is the ligand for the B61 receptor.

(Holzman et al., J. Am. Soc. Nephrol. 4, 466 (1993), Bartley et al., Nature 368, 558 (1994))

5 - endothelin, especially

- endothelin B

The promoter sequence was described by Benatti et al., J. Clin. Invest. 91, 1149 (1993).

- endothelin 1

10 The promoter sequence was described by Wilson et al., Mol. Cell. Biol. 10, 4654 (1990).

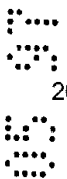
- endothelin receptors, in particular the endothelin B receptor

(Webb et al., Mol. Pharmacol. 47, 730 (1995), Haendler et al. J. Cardiovasc. Pharm. 20, 1 (1992)).



15 - mannose-6-phosphate receptors

The promoter sequences have been described by Ludwig et al. (Gene 142, 311 (1994), Oshima et al., (J. Biol. Chem. 263, 2553 (1988)) and Pohlmann et al. (PNAS USA 84, 5575 (1987)).



- von Willebrand factor

20 The promoter sequence was described by Jahroudi and Lynch (Mol. Cell. Biol. 14, 999 (1994)), Ferreira et al. (Biochem. J. 293, 641 (1993)) and Aird et al. (PNAS USA 92, 4567 (1995)).



- IL-1 α and IL-1 β

25 The promoter sequences were described by von Hangen et al., Mol. Carcinog. 2, 68 (1986), Turner et al., J. Immunol. 143, 3556 (1989), Fenton et al., J. Immunol. 138, 3972 (1987), Bensi et al., Cell Growth Diff. 1, 491 (1990), Hiscott et al., Mol. Cell. Biol. 13, 6231 (1993) and Mori et al., Blood 84, 1688 (1994).

- IL-1 receptor

30 The promoter sequence was described by Ye et al., PNAS USA 90, 2295 (1993).

- vascular cell adhesion molecule (VCAM-1)

The promoter sequence of VCAM-1 was described by Neish et al., Mol. Cell.

Biol. 15, 2558 (1995), Ahmad et al., J. Biol. Chem. 270, 8976 (1995), Neish et al., J. Exp. Med. 176, 1583 (1992), Iademarco et al., J. Biol. Chem. 267, 16323 (1992), and Cybulsky et al., PNAS USA 88, 7859 (1991).

- synthetic activator sequence

5 As an alternative to natural endothelium-specific promoters, synthetic activator sequences can also be used which comprise oligomerized binding sites for transcription factors which are preferentially or selectively active in endothelial cells. An example of this is the transcription factor GATA-2, whose binding site in the endothelin-1 gene is 5'-TTATCT-3' (Lee et al., Biol. Chem. 266, 16188 (1991), Dorfmann et al., J. Biol. Chem. 267, 1279 (1992) and Wilson et al., Mol. Cell Biol. 10, 4854 (1990)).

2.1.b) Selection of the promoters or activator sequences which are activated in cells in the vicinity of activated endothelial cells

In proliferating endothelia, neighboring cells are accessible, through opened tight junctions, for macromolecules derived from the blood. Because of the functional and anatomical interrelationships, the cells neighboring activated endothelial cells are target cells within the meaning of this invention.

- VEGF

The gene-regulatory sequences for the VEGF gene are

- the promoter sequence of the VEGF gene (5' flanking region) (Michenko et al., Cell. Mol. Biol. Res. 40, 35 (1994), Tischer et al., J. Biol. Chem. 266, 11947 (1991)) or
- the enhancer sequence of the VEGF gene (3' flanking region) (Michenko et al., Cell Mol. Biol. Res. 40, 35 (1994)) or
- the c-Src gene (Mukhopadhyay et al., Nature 375, 577 (1995), Bonham et al., Oncogene 8, 1973 (1993), Parker et al., Mol. Cell. Biol. 5, 831 (1985), and Anderson et al., Mol. Cell. Biol. 5, 112 (1985)) or
- the V-Src gene (Mukhopadhyay et al., Nature 375, 577 (1995), Anderson et al., Mol.

Cell. Biol. 5, 112 (1985) and Gibbs et al., J. Virol. 53, 19 (1985))

- steroid hormone receptors and their promoter elements (Truss and Beato, Endocr. Rev. 14, 459 (1993)), in particular the

- mouse mammary tumor virus promoter

5 The cDNA sequence of the promoter region of the long terminal repeat region of MMTV was described by Chalepakis et al., Cell 53, 371 (1988) and Truss and Beato (Endocr. Rev. 14, 459 (1993)).

2.2. Structural genes for antitumoral (or antiinflammatory) substances

10

2.2.a) Inhibitors of proliferation

An antitumoral or antiinflammatory substance within the meaning of this invention is to be understood as being the DNA sequence of a protein which inhibits the proliferation of endothelial cells. Examples of these DNA sequences are those for:

15

- retinoblastoma protein (pRb/p110) or for its analogs p107 and 120
- p53 protein
- p21 (WAF-1) protein
- p16 protein
- other CdK inhibitors
- GADD45 protein
- bak protein.

20

25 In order to prevent rapid intracellular inactivation of these cell-cycle inhibitors, those genes are preferably to be used which possess mutations for the inactivation sites of the expressed proteins, without the function of these proteins thereby being impaired.

30 The retinoblastoma protein (pRb) and the related p107 and p130 proteins are inactivated by phosphorylation. Consequently, a pRb/p110 cDNA sequence, p107 cDNA sequence or p130 cDNA sequence is preferably used which is point-mutated in such a way that the phosphorylation sites of the encoded protein are replaced

with non-phosphorylatable amino acids.

2.2.b) Coagulation-inducing factors and angiogenesis inhibitors

- 5 An antitumoral or antiinflammatory substance is additionally to be understood as being the DNA sequence for a protein which induces coagulation and/or inhibits angiogenesis. Examples of these proteins are:

- tissue factor (TF) and coagulation-active fragments thereof (Morrissey et al., Cell 50, 129 (1987), Scarpati et al., Biochem. 26, 5234 (1987), Spicer et al., PNAS USA 84, 5148 (1987), Rehemtulla et al., Thromb. Haemost. 65, 521 (1991))
- plasminogen activator inhibitor 1 (PAI-1)
- PAI-2
- PAI-3
- angiostatin
- interferons
 - IFN α
 - IFN β
 - IFN γ
- platelet factor 4
- IL-12
- TIMP-1
- TIMP-2
- TIMP-3
- leukemia inhibitory factor (LIF).

2.2.c) Cytostatic and cytotoxic proteins

- 30 However, an antitumoral or antiinflammatory substance is also to be understood as being a DNA sequence for a protein which exhibits, directly or indirectly, a cytostatic effect on tumors. These proteins include, in particular:

- antibodies or antibody fragments
- perforin
- granzyme
- IL-2
- 5 - IL-4
- IL-12
- interferons, for example
 - IFN α
 - IFN β
 - 10 • IFN γ
- TNF
 - TNF α
 - TNF β
- oncostatin M
- 15 - sphingomyelinase (Jarvis et al. PNAS - USA 91, 73, (1994))
- magainin and magainin derivatives (Cruciani et al. PNAS 88, 3792 (1991); Jacob et al. Ciba Found. Symp. 186, 197 (1994); Peck-Miller et al. Cancer Chemoth., Pharmac. 32, 109 (1993)).

2.2.d) Inflammation inducers

An antitumoral substance is additionally to be understood as being the DNA sequence for a protein which, where appropriate in addition to the antitumoral effect, stimulates inflammations and thereby contributes to the elimination of tumor cells.

25 Examples of these proteins are, in particular:

- RANTES (MCP-2)
- monocyte chemotactic and activating factor (MCAF)
- IL-8
- 30 - macrophage inflammatory protein 1 (MIP-1 α , - β)
- neutrophil activating protein 2 (NAP-2)
- IL-3
- IL-5

- human leukemia inhibitory factor (LIF)
- IL-7
- IL-11
- IL-13
- 5 - GM-CSF
- G-CSF
- M-CSF
- cobra venom factor (CVF) or constituent sequences of CVF which correspond functionally to human complement factor C3b, i.e. which can bind to
- 10 complement factor B and, after cleavage by factor D, constitute a C3 convertase. The DNA sequences for CVF and its constituent sequences were published by Fritzinger et al., Proc. Natl. Acad. Sci. USA 91, 12775 (1994).
- human complement factor C3 or its constituent sequence C3b. The DNA sequence for C3 and its constituent sequences was published by De Bruijn et al., Proc. Natl. Acad. Sci. USA 82, 708 (1985).
- 15 - cleavage products of human complement factor C3 which resemble CVF functionally and structurally. Cleavage products of this nature were described by O'Keefe et al., J. Biol. Chem. 263, 12690 (1988).
- bacterial proteins which activate complement or induce inflammations, such as porins of *Salmonella typhimurium* (Galdiero et al., Infection and Immunity 46, 559 (1984)), clumping factors of *Staphylococcus aureus* (Espersen Acta Path. Microb. et Imm. Scand. Sect C 93, 59 (1985)), modulins, particularly of Gram-negative bacteria (Henderson et al., Inflam. Res. 44, 187 (1995)), major outer membrane proteins of legionellas (Bellinger-Kawahara et al., J. Exp. Med. 172, 1201 (1990)) or of *Haemophilus influenzae* type B (Hetherington et al. Infection and Immunity 60, 19 (1992)) or of klebsiellas (Alberti et al., Infection and Immunity 61, 852 (1993)) or M molecules of group G streptococci (Campo et al., J. Infec. Dis. 171, 601 (1995)).
- 25
- 30 DNA sequences of fusion proteins formed between the listed cytokines or growth factors, on the one hand, and ligands for receptors on the cell membrane (for example an antibody which is specific for endothelial cells or tumor cells, or the Fc moiety of human immunoglobulin), on the other hand, can also be used as active

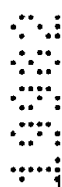
substance within the meaning of the invention. DNA sequences of this nature, and their preparation, were described, for example, in EP-A 0 464 633 A1.

2.2.e) Enzymes for activating precursors of cytostatic agents

5

However, an antitumoral or antiinflammatory substance is also to be understood as being the DNA sequence for an enzyme which is able to convert precursors of an antitumoral active compound into an antitumoral active compound.

10

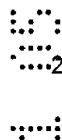


15

Enzymes of this nature, which cleave inactive precursor substances (prodrugs), thereby forming active cytostatic agents (drugs), and the relevant prodrugs and drugs in each case, have already been reviewed by Deonarain et al. (Br. J. Cancer 70, 786 (1994)), by Mullen, (Pharmac. Ther. 63, 199 (1994)) and by Harris et al. (Gene Ther. 1, 170 (1994)).



For example, use is to be made of the DNA sequence of one of the following enzymes:



20

- herpes simplex virus thymidine kinase
(Garapin et al., PNAS USA 76, 3755 (1979), Vile et al., Cancer Res. 53, 3860 (1993), Wagner et al., PNAS USA 78, 1441 (1981), Moelten et al., Cancer Res. 46, 5276 (1986), J. Natl. Cancer Inst. 82, 297 (1990))

- varicella zoster virus thymidine kinase
(Huber et al., PNAS USA 88, 8039 (1991), Snoeck, Int. J. Antimicrob. Agents 4, 211 (1994))

25

- bacterial nitroreductase
(Michael et al., FEMS Microbiol. Letters 125, 195 (1994), Bryant et al., J. Biol. Chem. 266, 4126 (1991), Watanabe et al., Nucleic Acids Res. 18, 1059 (1990))

30

- bacterial β -glucuronidase
(Jefferson et al., PNAS USA 83, 8447 (1986))
- plant β -glucuronidase from *Secale cereale*
(Schulz et al., Phytochemistry 26, 933 (1987))

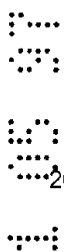
- human β -glucuronidase
(Bosslet et al., Br. J. Cancer 65, 234 (1992), Oshima et al., PNAS USA 84, 685 (1987))
- human carboxypeptidase (CB) e.g.
 - 5 • mast cell CB-A
(Reynolds et al., J. Clin. Invest. 89, 273 (1992))
 - pancreatic CB-B
(Yamamoto et al., J. Biol. Chem. 267, 2575 (1992), Catasus et al., J. Biol. Chem. 270, 6651 (1995))
 - 10 • bacterial carboxypeptidase
(Hamilton et al., J. Bacteriol. 174, 1626 (1992), Osterman et al., J. Protein Chem. 11, 561 (1992))
- bacterial β -lactamase
(Rodrigues et al., Cancer Res. 55, 63 (1995), Hussain et al., J. Bacteriol. 164, 223 (1985), Coque et al., EMBO J. 12, 631 (1993))
- bacterial cytosine deaminase
(Mullen et al., PNAS USA 89, 33 (1992), Austin et al., Mol. Pharmac. 43, 380 (1993), Danielson et al., Mol. Microbiol. 6, 1335 (1992))
- human catalase or peroxidase
(Ezurum et al., Nucl. Acids Res. 21, 1607 (1993))
- phosphatase, in particular
 - human alkaline phosphatase
(Gum et al., Cancer Res. 50, 1085 (1990))
 - human acid prostate phosphatase
(Sharieff et al., Am. J. Hum. Gen. 49, 412 (1991), Song et al., Gene 129, 291 (1993), Tailor et al., Nucl. Acids Res. 18, 4928 (1990))
 - 25 • type 5 acid phosphatase
(Gene 130, 201 (1993))
- oxidase, in particular
 - 30 • human lysyloxidase
(Kimi et al., J. Biol. Chem. 270, 7176 (1995))
 - human acid D-aminooxidase (Fukui et al., J. Biol. Chem. 267, 18631 (1992))

- peroxidase, in particular
 - human glutathione peroxidase
(Chada et al., Genomics 6, 268 (1990), Ishida et al., Nucl. Acids Res. 15, 10051 (1987))
 - human eosinophil peroxidase (Ten et al., J. Exp. Med. 169, 1757 (1989), Sahamaki et al., J. Biol. Chem. 264, 16828 (1989))
 - human thyroid peroxidase (Kimura, PNAS USA 84, 5555 (1987)).
- galactosidases

10 In order to facilitate secretion of the listed enzymes, the homologous signal sequence which is in each case contained in the DNA sequence can be replaced by a heterologous signal sequence which improves extracellular secretion.



Thus, for example, the signal sequence of β -glucuronidase (DNA position ≤ 27 to 93; Oshima et al., PNAS 84, 685 (1987)) can be replaced with the signal sequence for immunoglobulin (DNA position ≤ 63 to ≥ 107 ; Riechmann et al., Nature 332, 323 (1988)) or with the signal sequence for CEA (DNA position ≤ 33 to ≥ 134 ; Schrewe et al., Mol. Cell. Biol. 10, 2738 (1990), Berling et al., Cancer Res. 50, 6534 (1990)) or with the signal sequence of human respiratory syncytial virus glycoprotein (cDNA of amino acids ≤ 38 to ≥ 50 or 48 to 65; Lichtenstein et al., J. General Virol. 77, 109 (1996)).



In addition, preference is to be given to selecting DNAs of those enzymes which, as the result of point mutation, are stored to a lesser extent in lysosomes and secreted to a greater extent. Point mutations of this nature have been described for β -glucuronidase, for example (Shiplex et al., J.Biol.Chem. 268, 12193 (1993)).

A sequence for a transmembrane domain can be inserted, as an alternative to, or in addition to, the signal sequence, for the purpose of anchoring the enzyme into the cell membrane of the enzyme-forming cell.

Thus, for example, the transmembrane sequence of human macrophage colony-stimulating factor (DNA position ≤ 1485 to ≥ 1554 ; Cosman et al., Behring Inst. Mitt.

83, 15 (1988)) or the DNA sequence for the signal region and transmembrane region of human respiratory syncytial virus (RSV) glycoprotein G (amino acids 1 to 63 or their constituent sequences, amino acids 38 to 63; Vijaya et al., Mol. Cell Biol. 8, 1709 (1988), Lichtenstein et al., J. General Virol. 77, 109 (1996)) or the DNA sequence for the signal and transmembrane regions of influenza virus neuraminidase (amino acids 7 to 35 or the constituent sequence amino acids 7 to 27; Brown et al., J. Virol. 62, 3824 (1988)) can be inserted between the DNA sequence for the promoter and the DNA sequence for the enzyme (e.g. the β -glucuronidase).

10



The nucleotide sequence

GCCACC

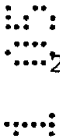
or

GCCGCC

15



can be inserted (Kozak, J. Cell. Biol. 108, 299 (1989) at the 3' end of the promoter, and directly prior to the 5' end of the start signal (ATG) of the signal or transmembrane sequence, for the purpose of augmenting translation.



20

However, the nucleotide sequence for a glycopospholipid anchor can also be inserted for the purpose of anchoring the enzyme in the cell membrane of the enzyme-forming cells.

25

A glycopospholipid anchor is inserted at the 3' end of the nucleotide sequence for the enzyme, and this insertion can be in addition to the insertion of a signal sequence.

30

Glycopospholipid anchors have been described, for example, for CEA (DNA position ≤ 893 to ≥ 1079 ; Berling et al., Cancer Res. 50, 6534 (1990)), and for N-CAM (Cunningham et al., Science 236, 799 (1987), and for other membrane proteins, such as Thy-1 (Clissold, Biochem. J. 281, 129 (1992)) or CD16 (Selvaray et al., Nature 333, 565 (1988)).

Ferguson et al. (Ann. Rev. Biochem. 57, 285 (1988)) have published a review of

glycophospholipid-anchored membrane proteins.

Another option for anchoring enzymes to the cell membrane in accordance with the present invention is to use a DNA sequence for a ligand-enzyme fusion protein. The specificity of the ligand of this fusion protein is directed against a membrane structure which is present on the cell membrane of proliferating endothelial cells or of tumor cells.

Ligands which bind to the surface of proliferating endothelial cells include, for example, antibodies or antibody fragments which are directed against membrane structures of endothelial cells, as described, for example, by Burrows et al. (Pharmac. Ther. 64, 155 (1994)), Hughes et al. (Cancer Res. 49, 6214 (1989)) and Maruyama et al. (PNAS-USA 87, 5744 (1990)). These antibodies include, in particular, antibodies against the VEGF receptors.

The murine monoclonal antibodies are preferably to be employed in humanized form. The humanization is effected in the manner described by Winter et al. (Nature 349, 293 (1991)) and Hoogenbooms et al. (Rev. Tr. Transfus. Hemobiol. 36, 19 (1993)). Antibody fragments are prepared in accordance with the state of the art, for example in the manner described by Winter et al. (Nature 349, 293 (1991)), Hoogenboom et al. (Ref. Tr. Transfus. Hemobiol. 36, 19 (1993); Girol. Mol. Immunol. 28, 1379 (1991) or Huston et al. (Intern. Rev. Immunol. 10, 195 (1993)).

The ligands furthermore include all active compounds which bind to membrane structures or membrane receptors on endothelial cells. For example, they include substances which contain mannose terminally, and, in addition, IL-1 or growth factors, or their fragments or constituent sequences thereof, which bind to receptors which are expressed by endothelial cells, such as PDGF, bFGF, VEGF, TGF β (Pusztain et al., J. Pathol. 169, 191 (1993)). In addition, they include adhesion molecules which bind to activated and/or proliferating endothelial cells. Adhesion molecules of this nature, such as SLe^x, LFA-1, MAC-1, LECAM-1 or VLA-4, have already been described (reviews in Augustin-Voss et al., J. Cell. Biol. 119, 483 (1992), Pauli et al., Cancer Metast. Rev. 9, 175 (1990) and Honn et al., Cancer

5

10

1	2	3	4	5
2	3	4	5	1
3	4	5	1	2
4	5	1	2	3
5	1	2	3	4

2020

Figure 6

25

30

5'-AGCTTCCGCATGCTGATGAGGCCGCAAGGCCGAAACGGCAG-3'

SEQ ID NO.: 2

5'-AATTCTGCCGTTTCGGCCTTGCGGCCTCATCAGCATGCGGA-3'

5 The DNA oligonucleotide sequence for another ribozyme for cleaving the cyclin D1 mRNA in the nucleotide position

(406) UUC CU GUC G CUG (417) (Xiong et al., 1991)

is, for example,

10

SEQ ID NO.: 3

5'-AGCTTCCAGCCTGATGAGGCCGCAAGGCCGAAACAGGAAG-3'

SEQ ID NO.: 4

5'-AATTCTTCCTGTTTCGGCCTTGCGGCCTCATCAGGCTGGA-3'

The DNA oligonucleotide sequence for a ribozyme which cleaves the mRNA of cyclin E (Koff et al., Cell 66, 1217 (1991)) in the nucleotide position

20

(208) GCA GUC UGU (216) (Xiong et al., 1991)

is, for example,

SEQ ID NO.: 5

25

5'-AGCTTGACACTGATGAGGCCGCAAGGCCGAAACTGCG-3'

SEQ ID NO.: 6

5'-AATTCGCAGTTTCGGCCTTGCGGCCTCATCAGTGTGCA-3'

30

The DNA oligonucleotide sequence for another ribozyme for cleaving the mRNA of cyclin E in the nucleotide position

(481) GAA GUC UAU (489) (Xiong et al., 1991)

is, for example,

SEQ ID NO.: 7

5'-AGCTTGAAGTTTATACTGATGAGGCCGCAAGGCCGAACTTCAG-3'

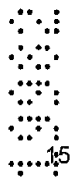
5

SEQ ID NO.: 8

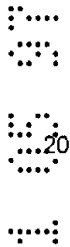
5'-AATTCTGAAGTTTCGGCCTTGCGGCCTCATCAGTATAAACTTCA-3'

2.3. Combination of several antitumoral or antiinflammatory substances

10



The invention furthermore relates to nucleic acid constructs in which there is a combination of the DNA sequences of several identical antitumoral or antiinflammatory substances (A,A) or of different antitumoral substances (A,B). In order to express two DNA sequences, the cDNA of an internal ribosome entry site (IRES) is preferably interposed as a regulatory element. This is illustrated by Figure 11.



IRES sequences of this nature were described, for example, by Mountford and Smith (TIG 11, 179 (1995), Kaufman et al., Nucl. Acids Res. 19, 4485 (1991), Morgan et al., Nucl. Acids Res. 20, 1293 (1992), Dirks et al., Gene 128, 247 (1993), Pelletier and Sonenberg, Nature 334, 320 (1988) and Sugitomo et al., BioTechn. 12, 694 (1994).

25

Thus, the cDNA of the IRES sequence of polio virus (position 140 to 630 of the 5' UTR; Pelletier and Sonenberg, Nature 334, 320 (1988)) can be used to link the DNA of the antiinflammatory substance A (at the 3' end) and the DNA of the antiinflammatory substance B (at the 5' terminus).

30

Depending on the combination, an active compound of this nature exhibits an additive (A+A, A+B1) or synergistic effect within the meaning of the invention.

3. Active compound for remedying deficient formation of blood cells

3.1. Selection of the promoters or the activator sequences for hematopoietic cells

Within the meaning of the present invention, a promoter or activator sequence composed of promoters or enhancers is preferably used which is a gene-regulatory sequence or an element from a gene which encodes a protein which is particularly strongly or selectively expressed in hematopoietic cells. Such gene-regulatory sequences include promoter sequences for genes of a cytokine or its receptor, whose expression in the immature hematopoietic cells (or in neighboring cells such as the stroma) precedes the subsequent cytokine, which exerts an effect on the hematopoietic cells and which is desired as an active substance. Examples of such cytokines which exert an effect on immature hematopoietic cells are:

- stem cell factor
- IL-1
- IL-3
- IL-6
- GM-CSF

3.2. Selection of the structural gene for active substances for hematopoietic cells

An active substance within the meaning of the invention is to be understood as being a DNA sequence whose expressed protein brings about proliferation and/or differentiation of blood cells.

4. Active compound for the therapy of autoimmune diseases, allergies and inflammations and for preventing organ rejections

4.1. Selection of the promoters or the activator sequences for inter alia autoimmune diseases

Gene-regulatory sequences of the genes for those proteins which are formed to an increased extent in macrophages and/or in lymphocytes during the immune reaction

are to be used as promoters or activator sequences composed of promoters or enhancers. Examples of proteins of this nature are:

- IL-1
- 5 - IL-1 β
- IL-1 receptor
- IL-2
- IL-2 receptor
- IL-3
- 10 - IL-3 receptor
- IFN γ
- IL-4
- IL-4 receptor
- IL-5
- 15 - IL-6
- LIF
- IL-7
- IL-10
- IL-11
- 20 - IL-12
- IL-13
- GM-CSF
- GM-CSF receptor
- integrin beta 2 proteins
- 25

4.2. Selection of the genes for active substances for inter alia autoimmune diseases

30 The active substance, within the meaning of the invention, is the DNA sequence for a cytokine, a chemokine, a growth factor, or one of their inhibitors, for a ribozyme which is catalytic for the transcription product of one of these DNA sequences or for the transcription product of a gene which encodes a cell-cycle control protein or a DNA sequence for an antibody or an antibody fragment or for an enzyme or for a

fusion protein of an antibody, cytokine or growth factor with an enzyme. The selection of the active substance depends on the underlying disease to be treated and on the promoter sequence which is selected.

5

5. Active compound for treating arthritis

5.1. Selection of the promoters or the activator sequences for arthritis

10



Within the meaning of the invention, promoters, activator sequences from promoters or enhancers or gene-regulatory sequences of those genes with which transcription factors interact in endothelial cells, synovial cells and inflammatory cells are preferably used. Within the meaning of this invention, the preferred promoter sequences include gene-regulatory sequences or elements from genes which encode proteins which are particularly expressed in endothelial cells, synovial cells and inflammatory cells.



5.2. Selection of the structural genes for active substances for arthritis



20



An active substance within the meaning of the invention is to be understood as being a DNA sequence whose expressed protein directly or indirectly inhibits inflammation, for example in a joint, and/or promotes the reconstitution of extracellular matrix (cartilage and connective tissue) in the joint.

25

6. Preparation of an active compound against infectious agents

The active compound can be prepared in two fundamentally different forms:

30

- for the therapy of virus infections and parasite invasions, or else
- for the prophylaxis of infectious diseases caused by viruses, bacteria or parasites.

Vaccines are used for the prophylaxis of infectious diseases. However, the possibilities of preparing effective vaccines in a conventional manner are limited (Brown, *Int. J. Technol. Assessm. Health Care* 10, 161 (1994)), Ellis, *Adv. Exp. Med. Biol.* 327, 263 (1992)) and Arnon et al., *FASEB J.* 6, 3265 (1992)).

5

As a consequence, the technology of DNA vaccines has been developed. However, these DNA vaccines raise questions with regard to degree of efficacy, safety and side effects (Fynan et al., *Int. J. Immunopharm.* 17, 79 (1995), Donnelly et al., *Immunol.* 2, 20 (1994)).

10

Active compounds for the prophylaxis of infectious diseases within the meaning of this invention are notable for a high degree of safety, due to their cell specificity and cell cycle regulation.



15

6.1. Selection of the promoters or activator sequences



6.1.a) For the therapy of infectious diseases



20

Promoter sequences of cell genes whose activity is particularly altered by infections with bacterial parasites, or promoter sequences of those viruses which transform the cells they infect and stimulate them to proliferate, are to be selected as activator sequences.



These viruses include, for example, HBV, HCV, HSV, HPV, HIV, EBV and HTLV.

25

6.2. Selection of the structural genes for active substances

6.2.a) For the therapy of infectious diseases

30

The DNA of a protein which exhibits cytostatic, cytotoxic or antiviral effects is to be selected as the active substance. Examples of cytotoxic or cytostatic proteins have already been listed above. When an enzyme is selected, the precursor which can be cleaved by this enzyme, and which is the precursor of an antiviral, cytotoxic or

antiparasitic substance, has to be administered subsequently.

Antivirally active cytokines and growth factors are also active substances for antiviral proteins within the meaning of this invention. They include, for example, the DNA sequences for the following active substances:

- IFN α
- IFN β
- IFN γ
- TNF β
- TNF α
- IL-1
- TGF β .

However, DNA sequences for protein fusions between the listed cytokines, growth factors, or the extracellular moiety of the receptors, on the one hand, and a ligand, on the other hand, can also be used as active substances within the meaning of the invention; for example, fusion proteins containing the Fc moiety of human immunoglobulin have been described in EP-A 0 464 633 A1.

Genes for ribozymes which digest the mRNA of genes for cell-cycle control proteins or the mRNA of viruses are also regarded as active substances. Ribozymes which are catalytic for HIV were reviewed, for example, by Christoffersen et al., J. Med. Chem. 38, 2033 (1995).

Within the meaning of this invention, an active substance is also the DNA sequence for an antibody of a specificity which inactivates the particular virus, or its VH-containing and VL-containing fragments, or its VH and VL fragments which are connected by way of a linker, and which is/are prepared, for example, in accordance with the methodology described by Marasco et al. (Proc. Natl. Acad. Sci. USA 90, 7889 (1993)) Examples of antibodies having such a specificity against viruses are listed in Section 8.4.

6.2.b) For the prophylaxis of infectious diseases

The DNA of a protein which is formed by the infectious agent and which leads, by means of triggering an immune reaction, i.e. by means of antibody binding and/or by means of the action of cytotoxic T lymphocytes, to the neutralization and/or destruction of the pathogen is to be selected as the active substance. So-called neutralization antigens of this nature are already being employed as vaccine antigens (see review in Ellis, Adv. Exp. Med. Biol. 327, 263 (1992)). Examples of DNA sequences which encode neutralization antigens can be obtained from the following publications:

- 
 - influenza A-virus antigen
 (Ulmer et al., Science 259, 1745 (1993), Robinson et al., Vaccine 11, 957 (1993), Fynan et al., Int. J. Immunopharmac. 17, 79 (1995))
- 
 - HIV antigens
 (Wang et al., PNAS USA 90, 4156 (1993))
- 
 - rabies virus antigen
 (Donnelly et al., Immunol. 2/1, 20 (1994))
- 
 - HSV (herpes simplex virus) antigen
 (Fleckenstein et al., Nature 274, 57 (1978))
- 
 - RSV (respiratory syncytial virus) antigen
 (Du et al., Bio/Tech. 12, 813 (1994), Hall, Science 265, 1393 (1993))
- 
 - parainfluenza virus antigen
 (Du et al., Bio/Techn. 12, 813 (1994))
- 
 - rotavirus antigen
 (Albert et al., J. Clin. Microbiol. 25, 183 (1987), Anderson et al., J. Infect. Dis. 153, 823 (1986), Battaglia et al., J. Infect. Dis. 155, 140 (1987), Chanock et al., J. Infect. Dis. 148, 49 (1983), Dyall-Smith et al., J. Virol. 38, 1099 (1981), Glass et al., Science 265, 1389 (1994))
- 
 - VZV (varicella zoster virus) antigen
 (Straus et al., Ann. Intern. Med. 109, 438 (1988), Gershon, Pediatr. Infect. Dis. 2, 171 (1991), Kinchington et al., J. Virol. 64, 4540 (1990))
- 
 - CMV (cytomegalovirus) antigen

- (Plotkin, Science 265, 1383 (1994))
- measles virus antigen
(Katz and Kellin, Science 265, 1391 (1994))
 - HPV (human papilloma virus) antigen
(Tindl and Frazer, Curr. Topics Microbiol. Immunol. 186, 217 (1994))
 - HBV (hepatitis B virus) antigen
(Valenzuela et al., Nature 280, 815 (1979), Heerman et al., J. Virol. 52, 396 (1984))
 - HCV (hepatitis C virus) antigen
(Cerny et al., Curr. Topics Microbiol. Immunol. 189, 169 (1994), Esteban et al., Progr. Liver Dis. 10, 253 (1992), Jung et al., Eur. J. Clin. Invest. 24, 641 (1994))
 - HDV (hepatitis D virus) antigen
(Iwarson, Scand. J. Infect. Dis. 24, 129 (1992), Consolo et al., Nephron. 61, 251 (1992))
 - HEV (hepatitis E virus) antigen
(Iwarson, Scand. J. Infect. Dis. 24, 129 (1992), Consolo et al., Nephron. 61, 251 (1992))
 - HAV (hepatitis A virus) antigen
(d'Hondt, Vaccine 10, 48 (1992), Andre, J. Infect. Dis. 171, 33 (1995), Lemon et al., Vaccine 10, 40 (1992), Melnick et al., Vaccine 10, 24 (1992), Flehmig, Baillieres Clin. Gastroenterol. 4, 707 (1990))
 - Vibrio cholerae antigen
(Levine and Kaper, Vaccine 11, 207 (1993))
 - Borrelia burgdorferi antigen
(Schaible et al., Immunol. Letters 36, 219 (1993), Wallich et al., Lab. Med. 17, 669 (1993))
 - Helicobacter pylori antigen
(Crabtree et al., Lancet 338, 332 (1991), Blaser, J. Infect. Dis. 161, 626 (1990), Cover and Blaser, J. Biol. Chem. 267, 10570 (1993), Cover et al., Infect. Immunol. 58, 603 (1990), Dunn et al., J. Biol. Chem. 265, 9464 (1990), Dunn et al., Infect. Immunol. 60, 1946 (1992), Lage et al., Acta Gastroenterol. Belg. 56 (suppl.), 61 (1993), Mobley et al., Scand. J. Gastroint. 26 (suppl.

187), 39 (1991))

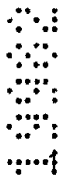
- malaria antigen

(Nussenzweig and Long, Science 265, 1381 (1994), Maurice, Science 267, 320 (1995), Enders et al., Vaccines 10, 920 (1992), Knapp et al., Infect. Imm. 60, 2397 (1992)).

5

However, within the meaning of the invention, active substances of this nature also include the DNA of an antiidiotypic antibody or its antigen-binding fragments, whose antigen-binding structures, the complementarity-determining regions, constitute copies of the protein or carbohydrate structure of the neutralization antigen of the infectious agent.

10



Antiidiotypic antibodies of this nature can, in particular, replace carbohydrate antigens in the case of bacterial infectious agents.

15



Antiidiotypic antibodies of this nature, and their cleavage products, have been reviewed by Hawkins et al (J. Immunotherapy 14, 273 (1993)) and Westerink and Apicella (Springer Seminars in Immunopathol. 15, 227 (1993)).



20

6.3. Combination of identical or different active substances for the therapy or prophylaxis of infectious diseases



The invention additionally relates to an active compound in which there is a combination of the DNA sequences of identical active substances (A,A) or different active substances (A,B). For the expression of two sequences, the cDNA of an internal ribosome entry site (IRES) is preferably interposed as a regulatory element.

25

30

IRES sequences of this nature have been described, for example, by Montford and Smith (TIG 11, 179 (1995), Kaufman et al., Nucl. Acids Res. 19, 4485 (1991), Morgan et al., Nucl. Acids Res. 20, 1293 (1992), Dirks et al., Gene 128, 247 (1993), Pelletier and Sonnenberg, Nature 334, 320 (1988) and Sugitomo et al., BioTechn. 12, 694 (1994).

Thus, the cDNA of the IRES sequence of polio virus (position 140 to 630 of the 5' UTR (Pelletier and Sonenberg, Nature 334, 320 (1988)) can be used to link the DNA of the antiviral substance A (at the 3' end) and the DNA of the antiviral substance B (at the 5' terminus). This is illustrated by Figure 12.

5

Depending on the combination, an active compound of this nature exhibits an additive (A+A, A+B1) or synergistic effect within the meaning of the invention.

10

Thus, two identical or two different antiviral active substances can be combined with each other for the therapy of virus diseases, for example.

15

In the prophylaxis of infectious diseases, several active substances, which encode different antigens of an infectious agent or of different infectious agents, can be combined with each other. Furthermore, the active substance which encodes the antigen of an infectious agent can be combined with an active substance which encodes a cytokine or a cytokine receptor.

20

25

The cytokines or cytokine receptors which are thereby formed (after injection of the active compound) at the same time as the infectious agent antigen can influence the nature and strength of the immune reaction which develops.

30

DNA sequences for cytokines and cytokine receptors which amplify the humoral immune reaction have already been described in 6.2.d), while those for cytokines and cytokine receptors which amplify the cellular immune reaction have been described in 6.2.a) and 6.2.c).

35

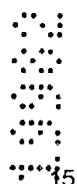
Examples of DNA sequences for cytokines which amplify the immune reaction as a whole are:

30

- IL-1 α
(Fenton, Int. J. Immunopharm. 14, 401 (1992), Furtani et al., Nucl. Acids Res. 14, 3167 (1986), Lafage et al., Blood 73, 104 (1989), March et al., Nature 315, 641 (1985))

- IL-1 β
(Bensi et al., Gene 52, 95 (1987), Auron et al., PNAS 81, 7907 (1984), Clark et al., Nucl. Acids Res. 14, 7897 (1986))
- IL-2
5 (Fletscher et al., Lymphok. Res. 6, 45 (1987), Matsui et al., Lymphokines 12, 1 (1985), Tanaguchi et al., Nature 302, 305 (1983))
- GM-CSF
(Gough et al., Nature 309, 763 (1984), Nicola et al., J. Biol. Chem. 254, 5290 (1979), Wong et al., Science 228, 810 (1985))

10



7. Active compound for treating leukemias and tumors

7.1. Selection of the promoters or activator sequences for leukemias



A gene-regulatory nucleotide sequence with which transcription factors interact which are formed or active in leukemia cells or tumor cells is intended as the promoter or activator sequence.



Within the meaning of this invention, the preferred promoters or activator sequences include gene-regulatory sequences or elements from genes which encode proteins which are formed particularly in leukemia cells or tumor cells.



7.2. Selection of the structural genes for active substances for leukemias

25

An active substance within the meaning of the invention is to be understood as being a DNA sequence whose expressed protein directly or indirectly inhibits the proliferation of cells, in particular also of leukemia cells or tumor cells. These active substances include, for example, the DNA sequences for inhibitory, cytostatic, apoptosis-inducing or cytotoxic proteins, or of enzymes, as have already been described.

30

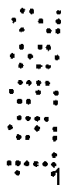
An active substance is furthermore to be understood as being the DNA sequence for

a ribozyme which catalyzes cleavage of the mRNA of the genes for cell-cycle control proteins.

- 5 8. Active compound for inhibiting the proliferation of smooth muscle cells in vascular occlusions

- 8.1. Selection of the promoters or the activator sequences for smooth muscle cells

- 10 Within the meaning of the invention, gene-regulatory sequences or elements from genes which encode proteins which are formed in particular in smooth muscle cells are preferably to be used as promoters or activator sequences composed of promoters or enhancers.



- 15 8.2. Selection of structural genes for active substances for smooth muscle cells



Within the meaning of the invention, an active substance is to be understood as being a DNA sequence whose expressed protein inhibits the proliferation of smooth muscle cells. These inhibitors of proliferation include the proteins which have already been mentioned in 2.2.a) and 2.2.c).



However, an active substance is also to be understood as being the DNA sequence for an enzyme which converts an inactive precursor of a cytostatic agent into a cytostatic agent (see 2.2.e)).

25

However, an active substance is also to be understood as being the DNA sequence for a ribozyme which is specific for the mRNA of genes for cell-cycle control proteins (see 2.2.f)).

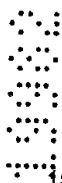
- 30 9. Active compound for inhibiting or promoting coagulation

9.1. Selection of the promoters or the activator sequences for inhibiting coagulation

5 Gene-regulatory sequences or elements from genes which encode proteins which can be detected in smooth muscle cells, in activated endothelial cells, in activated macrophages or in activated lymphocytes are preferably to be used as promoters or activator sequences within the meaning of the invention.

9.1.a) Smooth muscle cells

10



Examples of promoter sequences for genes in smooth muscle cells have already been mentioned.

9.1.b) activated endothelial cells

15



Examples of proteins which are formed in particular in activated endothelial cells have been described by Burrows et al. (Pharmac. Ther. 64, 155 (1994)). In particular, these proteins which occur to an increased extent in endothelial cells include, for example, those proteins which have already been listed above, as have the promoter sequences of their genes.



20



9.1.c) activated macrophages and/or activated lymphocytes

25

Within the meaning of this invention, activator sequences are also to be understood as being promoter sequences of the genes for proteins which are formed to an increased extent in macrophages and/or lymphocytes during the immune reaction. Proteins of this nature have already been listed.

30

9.2. Selection of the structural genes for active substances for inhibiting or promoting coagulation

A DNA sequence which encodes a protein which directly or indirectly inhibits thrombocyte aggregation or a blood coagulation factor, or stimulates fibrinolysis, is

to be used as an active substance for inhibiting coagulation within the meaning of this invention.

5 An active substance of this nature is designated as coagulation inhibitor. Genes for, for example, plasminogen activators (PA), for example tissue PA (tPA) or the urokinase-like PA (uPA) or protein C, antithrombin III, C-1S inhibitor, μ 1 antitrypsin, tissue factor pathway inhibitor (TFPI) or hirudin, are to be employed as coagulation inhibitors.

10 Within the meaning of this invention, a DNA sequence which encodes a protein which directly or indirectly promotes blood coagulation is to be used as an active substance for promoting coagulation. These proteins include, for example, blood coagulation factor VIII, blood coagulation factor IX or blood coagulation factor XIII.

15

10. Active compound for protecting against CNS damage

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10.1. Promoters or activator sequences composed of promoters or enhancers for an active compound for protecting against CNS damage

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10.1.a) Promoters or activator sequences which are activated in endothelial cells

30

These include, in particular, the promoter sequences for the genes of endothelial-cell-specific proteins.

35

10.1.b) Promoters or activator sequences which are activated in glia cells

A preferred activator sequence is additionally to be understood as being a nucleotide sequence (promoter sequence or enhancer sequence) which interacts with transcription factors which are formed to a particular extent, or are active, in glia cells.

40

10.2.) Selection of the structural genes for neurospecific factors

Within the meaning of the invention, a neurospecific factor is to be understood as being a DNA sequence which encodes a neuronal growth factor.

Examples for explaining the invention

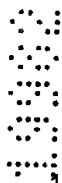
5

The following examples clarify the present invention.

•

Example 1

10

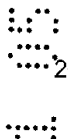


Preparation and testing of an activator-responsive promoter unit, with the activator-responsive promoter being the sequence for binding LexA.

The novel activator-responsive promoter unit is composed of the following different nucleotide sequences, which succeed each other in the downstream direction:



Activator subunit A



- the promoter of the *cdc25C* gene
(nucleic acids -290 to +121; Zwicker et al., EMBO J. 14, 4514 (1995); Zwicker et al., Nucl. Acids Res. 23, 3822 (1995))

25

- the cDNA for the DNA-binding domain of the LexA protein
(amino acids 1 to 81; Kim et al., Science 255, 203 (1992)) or the whole LexA protein (amino acids 1 to 202; Brent et al., Cell 43, 729 (1985))

- the cDNA for Gal 80
(amino acids 1 to 435; Leuther et al., Science 256, 1333 (1992))

30

Activator subunit B

- the promoter of the von Willebrand factor gene
(nucleic acids -487 to +247; Jahroudi and Lynch, Mol. Cell. Biol. 14, 999 (1994))

- the cDNA for the Gal80-binding domain of Gal4
(amino acids 851 to 881; Leuther et al., Science 256, 1333 (1992))
- the SV40 nuclear localization signal (NLS)
(SV40 large T; amino acids 126 to 132: PKKKRKV; Dingwall et al., TIBS 16, 478 (1991))
- the HSV-1 VP16 acid transactivating domain (TAD)
(amino acids 406 to 488; Triezenberg et al., Genes Developm. 2, 718 (1988); Triezenberg, Curr. Op. Gen. Developm. 5, 190 (1995))

Activator-responsive promoter

- the sequence for binding LexA (LexA operator) having the nucleotide sequence

SEQ ID NO.: 9

5'-TACTGTATGTACATACAGTA-3'

(Brent et al., Nature 612, 312 (1984)) which is coupled to the SV40 basal promoter (nucleic acids 48 to 5191; Tooze (ed.), DNA Tumor Viruses (Cold Spring Harbor New York, New York; Cold Spring Harbor Laboratory).

The order of the nucleotide sequences of the activator-responsive promoter units is depicted in Figure 13.

The activator sequence which has been described functions as follows:

- The cdc25C promoter regulates transcription of the combined cDNA's for the Lex DNA-binding protein and for Gal80 in a cell cycle-specific manner.
- The vWF promoter restricts transcription of the coupled cDNA for the Gal80-binding domain of Gal4, the SV40 NSL and the TAD to endothelial cells.
- The expression products of the activator subunits A and B dimerized by the

Gal80-binding domain of Gal4 being bound to Gal80.

The dimerization is depicted diagrammatically in Figure 14.

- 5 - The dimeric protein constitutes a chimeric transcription factor for the LexA operator activator-responsive promoter.

The promoter is now linked, at its 3' end, to the GCCACC sequence (Kocak, J. Cell. Biol. 108, 229 (1989)) and this latter is linked to the cDNA for the immunoglobulin signal peptide (nucleotide sequence ≤ 63 to ≥ 107 ; Riechmann et al., Nature 332, 323 (1988)). The cDNA for β -glucuronidase (nucleotide sequence ≤ 93 to ≥ 1982 ; Oshima et al., PNAS USA 84, 685 (1987)) is then joined to this sequence, as shown in Figure 15.

10
15 The nucleotide construct which has been prepared in this way is cloned into pUC18/19 or Bluescript-derived plasmid vectors, which are used, directly or in colloidal dispersion systems, for in-vivo administration.

20
25 The individual components of the construct are linked using suitable restriction sites, which are introduced at the termini of the different elements by way of PCR amplification. The linking is effected with the aid of enzymes which are specific for the restriction sites and which are known to the skilled person and with the aid of DNA ligases. These enzymes can be obtained commercially.

- 25 Human umbilical cord endothelial cells and fibroblasts (Wi-38) which are being maintained in culture are transfected with the described plasmid using a method known to the skilled person (Lucibello et al., EMBO J. 14, 132 (1995)) and the quantity of β -glucuronidase which is produced by the endothelial cells is measured using 4-methylumbelliferyl- β -glucuronide as the substrate.

30 In order to examine the cell-cycle specificity, endothelial cells are synchronized in G0/G1 by withdrawing methionine over a period of 48 hours (Nettelbeck et al., publication in preparation). The DNA content of the cells is determined in a

fluorescence-activated cell sorter after staining with Hoechst 33258 (Lucibello et al., EMBO J. 14, 132 (1995)).

The following results are obtained:

5

No increase in β -glucuronidase can be detected in transfected fibroblasts as compared with non-transfected fibroblasts.

10

Transfected endothelial cells express substantially more β -glucuronidase than do non-transfected endothelial cells.



15

Proliferating endothelial cells (DNA > 2 S) secrete substantially more β -glucuronidase than do endothelial cells which are synchronized in G0/G1 (DNA = 2 S).



Consequently the activator-responsive promoter unit which has been described, results in cell-specific, cell cycle-dependent expression of the β -glucuronidase structural gene.



20



25

Following local application, for example at the site of the tumor, or following intracranial or subarachnoid administration, or systemic, preferably intravenous or intraarterial, administration, an active compound according to the present invention has the effect of enabling, due to the cell-cycle specificity and endothelial-cell-specificity of the activator-responsive promoter unit, principally, if not exclusively, only proliferating endothelial cells to secrete β -glucuronidase. This β -glucuronidase cleaves a readily tolerated doxorubicin- β -glucuronide (Jacquesy et al., EPO 0511 917 A1), which is now injected, into doxorubicin, which has a cytostatic effect. The doxorubicin inhibits endothelial cell proliferation and has a cytostatic effect on these cells and also on adjacent tumor cells. This results in inhibition of tumor growth.

30

Since the active compound holds out the promise of a high degree of safety, due both to its cell specificity and its cell-cycle specificity, it can also be used for the therapy of tumor diseases at high doses and, if necessary, several times at intervals

of days or weeks.

Example 2

5

Preparation of a multiple promoter having a nuclear retention signal (NRS) and a nuclear export factor (NEF)

The novel multiple promoter is composed of the following different nucleotide sequences, which succeed each other in the downstream direction:

10



Element A

- the promoter of the *cdc25C* gene
(nucleic acids -290 to +121; Zwicker et al., EMBO J. 14, 4514 (1995); Zwicker et al., Nucl. Acids. Res. 23, 3822 (1995))
- the GCCACC sequence
(Kodak, J. Cell. Biol. 108, 229 (1989))
- the cDNA for the immunoglobulin signal peptide
(nucleotide sequence ≤ 63 to ≥ 107 ; Riechmann et al., Nature 332, 323 (1988))
- the cDNA for β -glucuronidase
(nucleotide sequence ≤ 93 to ≥ 1982 ; Oshima et al., PNAS USA 84, 685 (1987))
- 25 - the cDNA for HIV-1 virus RER as the nuclear retention signal (NRS)
(nucleotide sequence 7357 to 7602; Ratner et al., Nature 313, 277 (1985); Malim et al., Nature 338, 254 (1989)).

Element B

30

- the promoter of the von Willebrand factor (vWF) gene
(nucleic acids -487 to +247; Jahroudi and Lynch, Mol. Cell Biol. 14, 999, 1994))

- the cDNA for HIV-1 virus REV as the nuclear export factor (NEF)
(amino acid sequence 1-117; Ratner et al., Nature 313, 277 (1985)).

5 The nucleotide sequences of element A and element B are linked in accordance with the diagram in Figure 16.

The nucleotide construct which has been prepared in this way is cloned into pUC18/19 or Bluescript-derived plasmid vectors, which are used, directly or in colloidal dispersion systems, for in-vivo administration.

10



15

The individual components of the construct are linked using suitable restriction sites, which are introduced at the termini of the different elements by way of PCR amplification. The linking is effected with the aid of enzymes which are specific for the restriction sites and which are known to the skilled person and with the aid of DNA ligases. These enzymes can be obtained commercially.



20

Human umbilical cord endothelial cells and fibroblasts (Wi-38) which are being maintained in culture are transfected with the described plasmid using a method known to the skilled person (Lucibello et al., EMBO J. 14, 132 (1995)), and the quantity of β -glucuronidase which is produced by the endothelial cells is measured using 4-methylumbelliferyl- β -glucuronide as the substrate.



25

In order to examine the cell-cycle specificity, endothelial cells are synchronized in G0/G1 by withdrawing methionine over a period of 48 hours (Nettelbeck et al., publication in preparation). The DNA content of the cells is determined in a fluorescence-activated cell sorter after staining with Hoechst 33258 (Lucibello et al., EMBO J. 14, 132 (1995)).

The following results are obtained:

30

No increase in β -glucuronidase can be detected in transfected fibroblasts as compared with non-transfected fibroblasts.

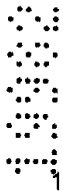
Transfected endothelial cells express substantially more β -glucuronidase than do

non-transfected endothelial cells.

Proliferating endothelial cells (DNA > 2 S) secrete substantially more β -glucuronidase than do endothelial cells which are synchronized in G0/G1 (DNA = 2 S).

Consequently, the multiple promoter unit which has been described results in cell-specific, cell cycle-dependent expression of the β -glucuronidase structural gene.

10



Example 3

Preparation of an activator-responsive promoter unit in which the activator-responsive promoter is the sequence for binding Gal4.

15



The novel activator-responsive promoter unit is composed of the following different nucleotide sequences, which succeed each other in the downstream direction:



Activator subunit A

20



25

- the promoter of the cdc25C gene
(nucleic acids -290 to +121; Zwicker et al., EMBO J. 14, 4514 (1995); Zwicker et al., Nucl. Acids Res. 23, 3822 (1995))
- the cDNA for the DNA-binding domain of the Gal4 protein
(amino acids 1 to 147; Chasman and Kornberg, Mol. Cell Biol. 10, 2916 (1990))
- the cDNA for Gal80
(amino acids 1 to 435; Leuther et al., Science 256, 1333 (1992))

30

Activator subunit B

- the promoter of the von Willebrand factor gene
(nucleic acids -487 to +247; Jahroudi and Lynck, Mol. Cell. Biol. 14, 999

(1994))

- the cDNA for the Gal80-binding domain of Gal4
(amino acids 851 to 881; Leuther et al., Science 256, 1333 (1992))
- the SV40 nuclear localization signal (NLS)
(SV40 large T; amino acids 126 to 132: PKKKRKV; Dingwall et al., TIBS16, 478 (1991))
- the HSV-1 VP16 acid transactivating domain (TAD)
(amino acids 406 to 488; Triezenberg et al., Genes Developm. 2, 718 (1988); Triezenberg, Curr. Opin. Gen. Developm. 5, 190 (1995))

10



Activator-responsive promoter

- the sequence for binding Gal4, having the nucleotide sequence
5'-CGGACAACTGTTGAC CG-3' (Chasman and Kornberg, Mol. Cell Biol. 10, 2916 (1999)), which is coupled to the SV40 basal promoter (nucleic acids 48 to 5191; Tooze (ed.), DNA Tumor Viruses (Cold Spring Harbor New York, New York; Cold Spring Harbor Laboratory).



The order of the nucleotide sequences of the activator-responsive promoter units is depicted in Figure 17.



The activator sequence which has been described functions as follows:

- The cdc25C promoter regulates transcription of the combined cDNA's for the Gal4-binding protein and for Gal80 in a cell cycle-specific manner.
- The promoter of the vWF gene restricts transcription of the coupled cDNA for the Gal80-binding domain of Gal4, the SV40 NSL and the TAD to endothelial cells.
- The expression products of the activator subunits A and B dimerize by the Gal80-binding domain of Gal4 being bound to Gal80.

The dimerization is depicted diagrammatically in Figure 18.

- The dimeric protein constitutes a chimeric transcription factor for the activator-responsive promoter (DNA sequence for the Gal4-binding domain/SV40 promoter).

5 The promoter is now linked, at its 3' end, to the GCCACC sequence (Kocak, J. Cell Biol. 108, 229 (1989)), and this latter is linked to the cDNA for the immunoglobulin signal peptide (nucleotide sequence ≤ 63 to ≥ 107 ; Riechmann et al., Nature 332, 323 (1988)). The cDNA for β -glucuronidase (nucleotide sequence ≤ 93 to ≥ 1982 ; Oshima et al., PNAS USA 84, 685 (1987)) is joined to this sequence, as shown in Figure 19.



The nucleotide construct which has been prepared in this way is cloned into pUC18/19 or Bluescript-derived plasmid vectors, which are used, directly or in colloidal dispersion systems, for in-vivo administration.



The individual components of the construct are linked using suitable restriction sites, which are introduced at the termini of the different elements by way of PCR amplification. The linking is effected with the aid of enzymes which are specific for the restriction sites and which are known to the skilled person and with the aid of DNA ligases. These enzymes can be obtained commercially.



Human umbilical cord endothelial cells and fibroblasts (Wi-38) which are being maintained in culture are transfected with the described plasmid using a method known to the skilled person (Lucibello et al., EMBO J. 14, 132 (1995)), and the quantity of β -glucuronidase which is produced by the endothelial cells is measured using 4-methylumbelliferyl- β -glucuronide as the substrate.

30 In order to examine the cell-cycle specificity, endothelial cells are synchronized in G0/G1 by withdrawing methionine over a period of 48 hours (Nettelbeck et al., publication in preparation). The DNA content of the cells is determined in a fluorescence-activated cell sorter after staining with Hoechst 33258 (Lucibello et al., EMBO J. 14, 132 (1995)).

The following results are obtained:

No increase in β -glucuronidase can be detected in transfected fibroblasts as compared with non-transfected fibroblasts.

5

Transfected endothelial cells express substantially more β -glucuronidase than do non-transfected endothelial cells.

10

Proliferating endothelial cells (DNA >2 S) secrete substantially more β -glucuronidase than do endothelial cells which are synchronized in G0/G1 (DNA = 2 S).

Consequently, the activator-responsive promoter unit which has been described results in cell-specific, cell cycle-dependent expression of the β -glucuronidase structural gene.

15

Example 4

20

Preparation and testing of another activator-responsive promoter unit, in which the activator-responsive promoter is the sequence for binding Gal4.

This novel activator-responsive promoter unit is composed of the following different nucleotide sequences, which succeed each other in the downstream direction:

25

Activator subunit A

30

- the promoter of the cdc25C gene (nucleic acids -290 to +121; Zwicker et al., EMBO J. 14, 4514 (1995); Zwicker et al., Nucl. Acids Res. 23, 3822 (1995))
- the SV40 nuclear localization signal (NLS) (SV40 large T, amino acids 126-132; PKKKRKV, Dingwall et al., TIBS 16, 478 (1991))
- the HSV-1 VP16 acid transactivating domain (TAD) (amino acids 406 to 488; Triezenberg et al., Genes Developm. 2, 718 (1988); Triezenberg, Curr. Opin.

Gen. Developm. 5, 190 (1995))

- the cDNA for the cytoplasmic moiety of the CD4 glycoprotein (amino acids 397-435; Simpson et al., Oncogene 4, 1141 (1989); Maddon et al., Cell 42, 93 (1985))

5

Activator subunit B

- the promoter of the cdc25C gene (nucleic acids -290 to +121; Zwicker et al., EMBO J. 14, 4514 (1995); Zwicker et al., Nucl. Acids Res. 23, 3822 (1995))
- the SV40 nuclear localization signal (NLS) (SV40 large T; amino acids 126-132 PKKKRKV; Dingwall et al., TIBS 16, 478 (1991))
- the cDNA for the DNA-binding domain of the Gal4 protein (amino acids 1 to 147, Chasman and Kornberg, Mol. Cell. Biol. 10, 2916 (1990))
- the cDNA for the CD4-binding sequence of the p56 lck protein (amino acids 1-71; Shaw et al., Cell 59, 627 (1989); Turner et al., Cell 60, 755 (1990); Perlmutter et al., J. Cell. Biochem. 38, 117 (1988))

Activator-responsive promoters

- 10x the binding sequence for Gal4-binding protein having the nucleotide sequence 5'-CGGACAATGTTGACCG-3' (Chasman and Kornberg, Mol. Cell. Biol. 10, 2916 (1989))
- the SV40 basal promoter (nucleic acids 48 to 5191; Tooze (ed), DNA Tumor Viruses (Cold Spring Harbor New York, New York, Cold Spring Harbor Laboratory)

Effector gene

- the cDNA for luciferase (reporter gene) (Nordeen, BioTechniques 6, 454 (1988))

The activator sequence which has been described functions as follows:

- The cdc25C promoter regulates transcription of the combined cDNA's for the activating domain of VP16 and the cytoplasmic moiety of CD4 (activating subunit A) in a cell cycle-specific manner.
- The cdc25C promoter additionally regulates transcription of the combined cDNA's for the DNA-binding protein of Gal4 and the CD4-binding moiety of the p56 lck protein (activating subunit B).
- The expression products of activator subunits A and B dimerize by the CD4 domain being bound to the p56 lck domain.
- The dimeric protein constitutes a chimeric transcription factor for the activator-responsive promoter (DNA sequence for the Gal4-binding domain/SV40 promoter) for transcription of the effector gene (= reporter gene = luciferase gene).

The nucleotide construct which has been prepared in this way is cloned into the pXP2 plasmid vector (Nordeen, BioTechniques 6, 454 (1988)), which is used, directly or in colloidal dispersion systems, for in-vivo administration.

The individual components of the construct are linked using suitable restriction sites, which are introduced at the termini of the different elements by way of PCR amplification. The linking is effected with the aid of enzymes which are specific for the restriction sites and which are known to the skilled person and with the aid of DNA ligases. These enzymes can be obtained commercially.

3T3 fibroblasts which are being maintained in culture are transfected with the described plasmid using a method known to the skilled person (Lucibello et al., EMBO J. 14, 132 (1995)), and the quantity of luciferase which is produced by the fibroblasts is measured as described by Herber et al. (Oncogene 9, 1295 (1994)) and Lucibello et al. (EMBO J. 14, 132 (1995)).

In order to examine the cell-cycle specificity, the fibroblasts are synchronized in G0/G1 by withdrawing serum over a period of 48 hours. The DNA content of the cells is determined in a fluorescence-activated cell sorter after staining with Hoechst 33258 (Lucibello et al., EMBO J. 14, 132 (1995)).

The following results are obtained:

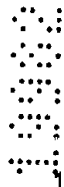
A marked increase in luciferase can be detected in transfected fibroblasts as compared with non-transfected fibroblasts.

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Proliferating fibroblasts (DNA > 2 S) form substantially more luciferase than do fibroblasts which are synchronized in G0/G1 (DNA = 2 S).

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Consequently, the activator-responsive promoter unit which has been described results in cell cycle-dependent expression of the luciferase reporter gene.



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Following local application, for example at the site of the tumor, or following intracranial or subarachnoid administration, or systemic, preferably intravenous or intraarterial, administration, an active compound according to the present invention has the effect of enabling, due to the cell-cycle specificity and endothelial-cell specificity of the activator-responsive promoter unit, principally, if not exclusively, only proliferating endothelial cells to secrete β -glucuronidase. This β -glucuronidase cleaves readily tolerated doxorubicin- β -glucuronide (Jacquesy et al., EPO 0 511 917 A1), which is now injected, into doxorubicin, which has a cytostatic effect. The doxorubicin inhibits endothelial cell proliferation and has a cytostatic effect on these cells, and also on adjacent tumor cells. This results in inhibition of the growth of the tumor.

Figure Legends:

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Figure 1: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

Figure 2: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

Figure 3: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

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Figure 4: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

Figure 5: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

Figure 6: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

5 Figure 7: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

Figure 8: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

Figure 9: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

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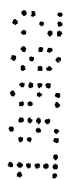


Figure 10: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

Figure 11: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

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Figure 12: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

Figure 13: Self-explaining scheme illustrating an nucleic acid construct of the present invention.



Figure 14: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

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Figure 15: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

Figure 16: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

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Figure 17: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

Figure 18: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

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Figure 19: Self-explaining scheme illustrating an nucleic acid construct of the present invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Hoechst Aktiengesellschaft
(B) STREET: -
(C) TOWN: Frankfurt
(D) STATE: -
(E) COUNTRY: Germany
(F) POST CODE (ZIP): 65926
(G) TELEPHONE: 069-305-3005
(H) TELEFAX: 069-35-7175
(I) TELEX: 412 34 700 ho d

(ii) TITLE OF INVENTION: Nucleic acid constructs containing genes encoding transport signals

(iii) NUMBER OF SEQUENCES: 9

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURES:

(A) NAME/KEY: exon
(B) LOCATION: 1..41

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AGCTTCCGCA TGCTGATGAG GCCGCAAGGC CGAAACGGCA G

41

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURES:

(A) NAME/KEY: exon
(B) LOCATION: 1..41

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURES:

- (A) NAME/KEY: exon
- (B) LOCATION: 1..40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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(2) INFORMATION FOR SEQ ID NO: 4:

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 - (B) TYPE: nucleic acid
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 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURES:

- (A) NAME/KEY: exon
- (B) LOCATION: 1..40

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 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURES:

- (A) NAME/KEY: exon
- (B) LOCATION: 1..38

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

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38

(2) INFORMATION FOR SEQ ID NO: 6:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 38 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

- (ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..38

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

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38



(2) INFORMATION FOR SEQ ID NO: 7:

- (i) SEQUENCE CHARACTERISTICS:
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 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

- (ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..44



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

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44



(2) INFORMATION FOR SEQ ID NO: 8:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 44 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

- (ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..44

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AATTCTGAAG TTTCGGCCTT GCGGCCTCAT CAGTATAAAC TTCA

44

(2) INFORMATION FOR SEQ ID NO: 9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

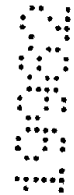
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 - (B) LOCATION: 1..20

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

TACTGTATGT ACATACAGTA

20

The terms "comprise", "comprises", "comprised" and "comprising" when used in this specification are taken to specify the presence of stated features, integers, steps or components but does not preclude the presence or addition of one or more other features, integers, steps components or groups thereof.



THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A nucleic acid construct, which possesses the following components, in the reading direction, from the 5' end to the 3' end:
 - a) a first nonspecific, cell-specific, virus-specific, metabolically and/or cell-cycle-specifically activatable promoter sequence or enhancer sequence which activates the basal transcription of a transgene,
 - b) a transgene which, as a structural gene, encodes an active compound,
 - c) a nuclear retention signal whose cDNA is linked, directly or indirectly, at the 5' end, to the 3' end of the structural gene,
 - d) an additional promoter sequence or enhancer sequence which activates the basal transcription of a nuclear export factor, and
 - e) a nucleic acid which encodes a nuclear export factor which binds to the transcription product of the nuclear retention signal (c) and thereby mediates transport of the transcription product of the transgene out of the cell nucleus into the cytoplasm.
2. A nucleic acid construct as claimed in claim 1, wherein the transcription product of the nuclear retention signal possesses a structure for binding a nuclear export factor.
3. A nucleic acid construct as claimed in claim 1 or 2, wherein the first promoter



sequence or enhancer sequence (a) and the second promoter sequence or enhancer sequence (d) are identical or different, and wherein at least one of the components a) and d) is nonspecifically, cell-specifically, virus-specifically, metabolically, in particular by hypoxia, or cell-cycle-specifically activatable.

4. A nucleic acid construct as claimed in claim 3, wherein at least one of the promoter sequences or enhancer sequences (a) and (d) is a chimeric promoter in which the promoter module CDE-CHR or E2FBS-CHR interacts with an adjacent, upstream, cell-specifically, virus-specifically or metabolically activatable activator sequence and can thereby influence, in particular inhibit, the expression of a downstream gene.

5. A nucleic acid construct as claimed in claim 1 or 2, wherein at least one of the components a) and d) constitutes an activator-responsive promoter unit comprising the following components:

- f) at least one promoter sequence or enhancer sequence which is non-specifically, virus-specifically, metabolically, cell-specifically and/or cell-cycle-specifically activatable,
- g) at least one activator subunit which is located downstream of the promoter sequence or enhancer sequence (f) and is activated, in its basal transcription, by the promoter sequence or enhancer sequence (f), and
- h) an activator-responsive promoter which is activated by the expression products of one activator subunit (g) or several identical or different activator subunits (g, g').

6. A nucleic acid construct as claimed in claim 5, in which the promoter sequence or enhancer sequence (a) and/or (d) and/or the activator-responsive promoter is a chimeric promoter and the activator subunit (g) is a gene for at least one transcription factor which activates the chimeric promoter of the activator-responsive promoter (h).

7. A nucleic acid construct as claimed in claim 5, wherein

- the activator-responsive promoter (h) is the LexA operator in combination with the SV40 promoter, and
- the activator subunit (g) comprises the cDNA for the LexA DNA-binding protein encoding amino acids 1-81 or 1-202, whose 3' end is linked to the 5' end of the cDNA for the Gal80 protein (amino acids 1-435), and
- a further activator subunit (g') contains the cDNA of the Gal80-binding domain of the Gal4 protein encoding amino acids 851-881, whose 3' end is linked to the 5' end of the cDNA of the SV40 large T antigen encoding amino acids 126-132, whose 3' end is linked to the 5' end of the cDNA for the HSV-1 VP16 transactivating domain encoding amino acids 406-488.
- or wherein the activator-responsive promoter (h) contains one or more sequences for binding the Gal4 protein in combination with at least one SV40 promoter and the activator subunits (g, g') either contain:
 - activator subunit g: the cDNA for the DNA-binding domain of the Gal4 protein (amino acids 1 to 147) and the cDNA for Gal80, and the
 - activator subunit g': the cDNA for the Gal80-binding domain of Gal4 (amino acids 851 to 881); the cDNA for the SV40 nuclear localization signal (NLS) (amino acids 126 to 132) and the cDNA for the HSV-1 VP16 acid transactivating domain (amino acids 406 to 488)
- or else contain
 - activating subunit g: the cDNA for the SV40 nuclear localization signal (amino acids 126-132); the cDNA for the HSV-1 VP16 acid transactivating domain (amino acids 406 to 488) and the cDNA for the cytoplasmic moiety of the CD4 glycoprotein (amino acids 397-435), and the
 - activating subunit g': the cDNA for the SV40 nuclear localization signal (amino acids 126-132), the cDNA for the DNA-binding domain of the Gal4 protein (amino acids 1 to 147) and the cDNA for the CD4-binding sequence of the p56 lck protein (amino acids 1-71).

8. A nucleic acid construct as claimed in one of the preceding claims, wherein the gene encoding the nuclear retention signal (c) is selected from the group

consisting of the rev-responsive element (RRE) of HIV-1 or HIV-2, the RRE-equivalent retention signal of retroviruses or the RRE-equivalent retention signal of HBV.

9. A nucleic acid construct as claimed in claim 1 or 2, wherein the nuclear export factor (e) is a gene which is selected from the group consisting of the rev gene of the viruses HIV-1, HIV-2, visna-maedi virus, Caprine arthritis encephalitis virus, equine infectious anemia virus, feline immunodeficiency virus, of retroviruses, of HTLV, or the gene of the hnRNP-A1 protein or the gene of the transcription factor TFIII-A.

10. A nucleic acid construct as claimed in any one of the preceding claims, wherein the nucleic acid is DNA.

11. A nucleic acid construct as claimed in any one of the preceding claims, wherein the nucleic acid construct is a vector.

12. A nucleic acid construct as claimed in claim 11, which is a plasmid vector.

13. A nucleic acid construct as claimed in claim 11, which is a viral vector.

14. A nucleic acid construct as claimed in one of the preceding claims, wherein the transgene (b) is a structural gene which encodes an active compound which is selected from the group consisting of cytokines, growth factors, antibodies or antibody fragments, fusion proteins composed of a ligand and an enzyme, a cytokine or growth factor, receptors for cytokines or growth factors, proteins having an antiproliferative, apoptotic or cytotoxic effect, angiogenesis inhibitors and/or thrombosis-inducing proteins, blood coagulation factor, coagulation inhibitors, fibrinolysis-inducing protein, complement-activating proteins such as cobra venom factor; human C3b, modified C3b; bacterial proteins, virus coat proteins, bacterial antigens and parasitic antigens, peptides or proteins having an effect on the blood circulation, and ribozymes.

15. A nucleic acid construct as claimed in claim 14, wherein the transgene (b) is a structural gene which encodes a ribozyme which inactivates the mRNA which encodes a protein selected from the group consisting of cell-cycle control proteins, in particular cyclin A, cyclin B, cyclin D1, cyclin E, E2F1-5, cdc2, cdc25C or DP1, virus proteins or cytokines or growth factors or their receptors.

16. A nucleic acid construct as claimed in one of the preceding claims, wherein the promoter sequence, enhancer sequence or activator sequence is selected from the group consisting of gene-regulatory nucleotide sequences which are activated in endothelial cells, smooth muscle cells, striated muscle cells, macrophages, lymphocytes, tumor cells, liver cells, leukemia cells and glia cells, or of promoter sequences of the HBV, HCV, HSV, HPV, EBV, HTLV or HIV viruses .



17. A nucleic acid construct as claimed in one of the preceding claims, wherein the transgene (b) is a structural gene which encodes an enzyme which cleaves a precursor of a drug, thereby forming a drug.



18. A nucleic acid construct as claimed in one of the preceding claims, wherein the transgene b) is a structural gene which encodes a ligand-enzyme fusion protein.

19. A nucleic acid construct as claimed in claim 18, wherein the ligand binds to proliferating endothelial cells and is selected from a group consisting of antibodies or their fragments, terminal mannose-containing proteins, cytokines such as IL-1 or TNF, growth factors such as PDGF, G-FGF, VEGF, TGF β , or adhesion molecules such as SLex, LFA-1, MAC-1, LECAM-1 or VLA-1.

20. A nucleic acid construct as claimed in claim 18, wherein the ligand binds to tumor cells.

21. An isolated cell, which harbors a nucleic acid construct as claimed in one of claims 1 to 20.



22. The use of a nucleic acid construct as claimed in any one of claims 1 to 20, or of a cell as claimed in claim 21, for preparing a medicine for treating a disease.

23. The use of a nucleic acid construct as claimed in any one of claims 1 to 20, or of an isolated cell as claimed in claim 21, for the preparation of the medicine comprising the insertion of the nucleic acid construct into a target cell, for treating a disease.

24. The use as claimed in claim 23, wherein the disease is accompanied by excessive cell multiplication.



25. The use as claimed in any one of claims 22 to 24, wherein the preparation of the medicine comprises the conversion of a nucleic acid construct, as claimed in any one of claims 1 to 20, into a form which makes it possible to introduce the nucleic acid construct into the target cell.



26. A method of treatment of disease comprising administration of a nucleic acid construct as claimed in any one of claims 1 to 20, or of a cell as claimed in claim 21.

27. A method of treatment of disease comprising administration of a nucleic construct as claimed in any one of claims 1 to 20, or of an isolated cell as claimed in claim 21, wherein the nucleic acid construct is inserted into a target cell.

28. The method of claim 27, wherein the disease is accompanied by excessive cell multiplication.



29. The method as claimed in any one of claims 26 to 28, wherein the method includes the conversion of a nucleic acid construct, as claimed in any one of claims 1 to 20, into a form which makes it possible to introduce the nucleic acid construct into the target cell.

HOECHST AKTIENGESELLSCHAFT

DATED this 2nd day of December, 1999



WATERMARK PATENT & TRADEMARK ATTORNEYS
290 BURWOOD ROAD
HAWTHORN VIC 3122
AUSTRALIA



KJS:KMH:MXM



Figure 1

1/8

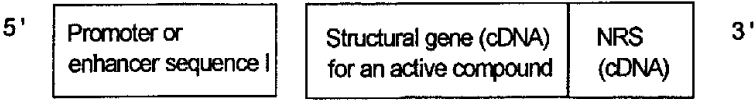


Figure 2

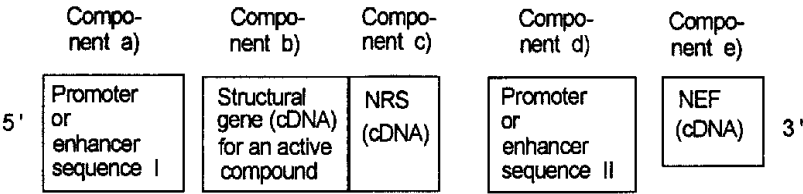


Figure 3

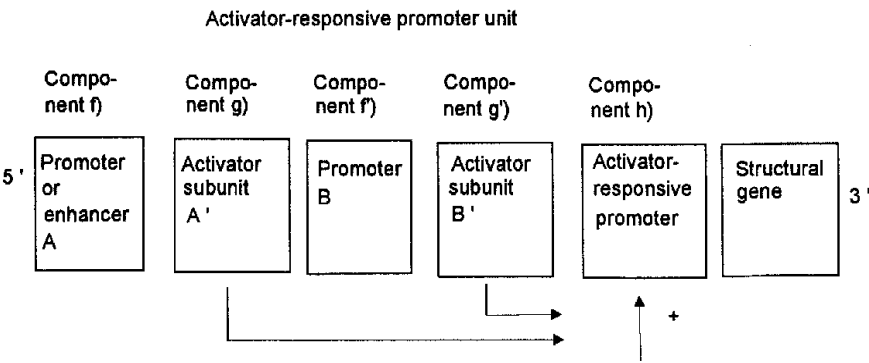


Figure 4

2/8

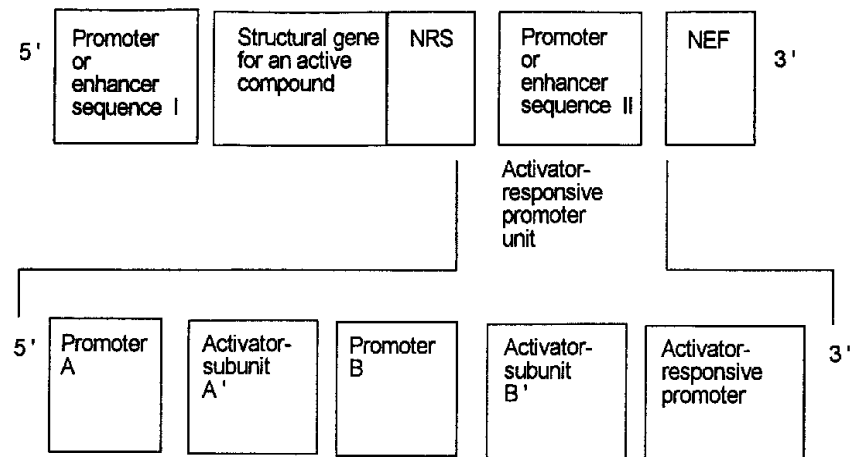


Figure 5

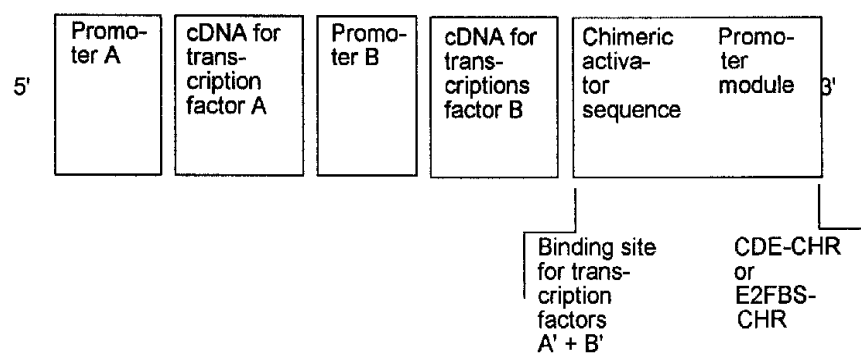


Figure 6

3/8

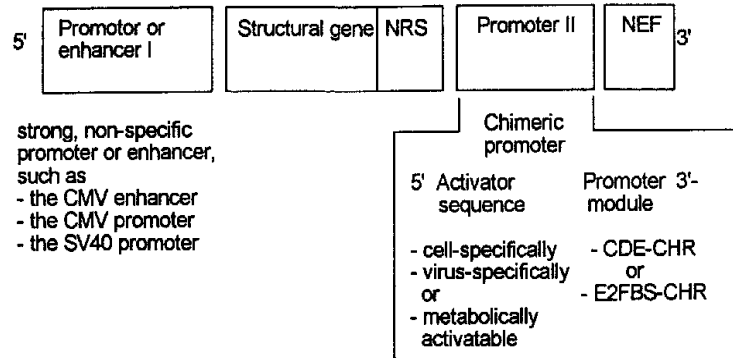


Figure 7

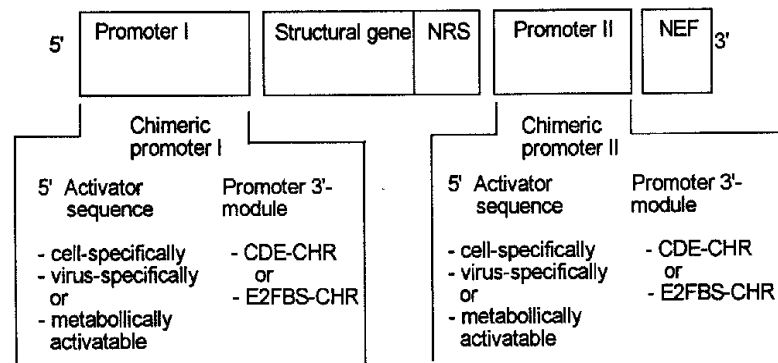


Figure 8

4/8

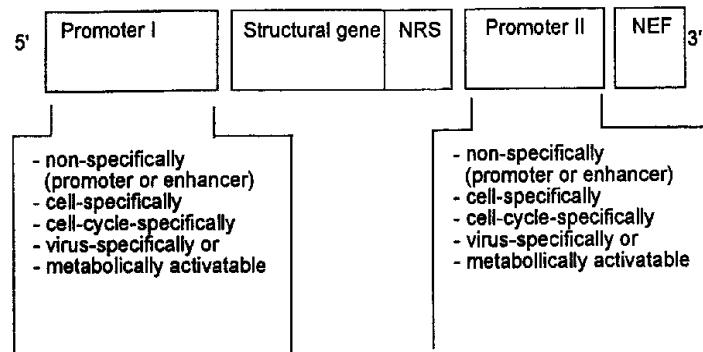


Figure 9

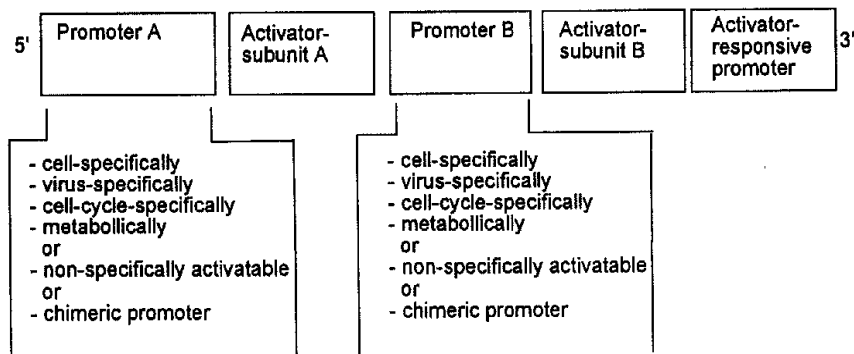


Figure 10

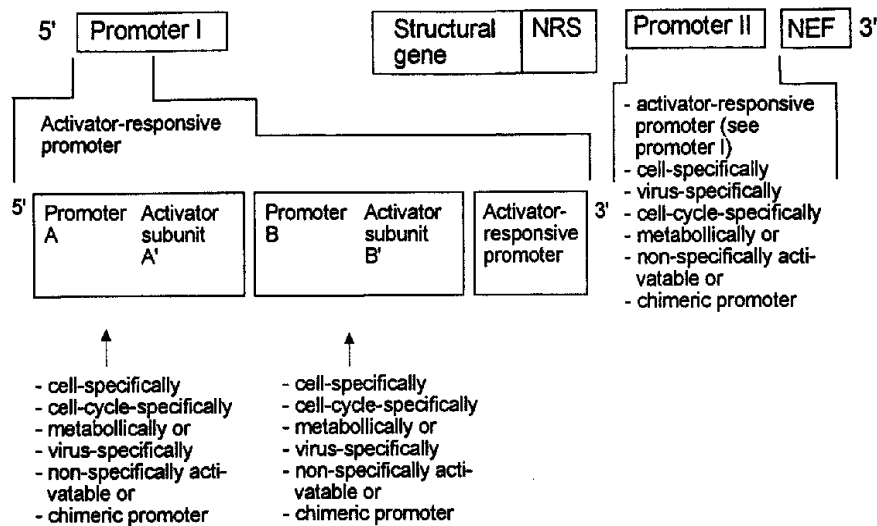


Figure 11

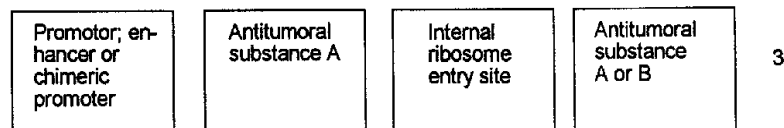


Figure 12

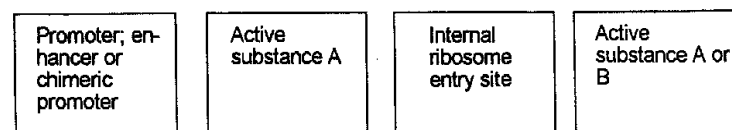
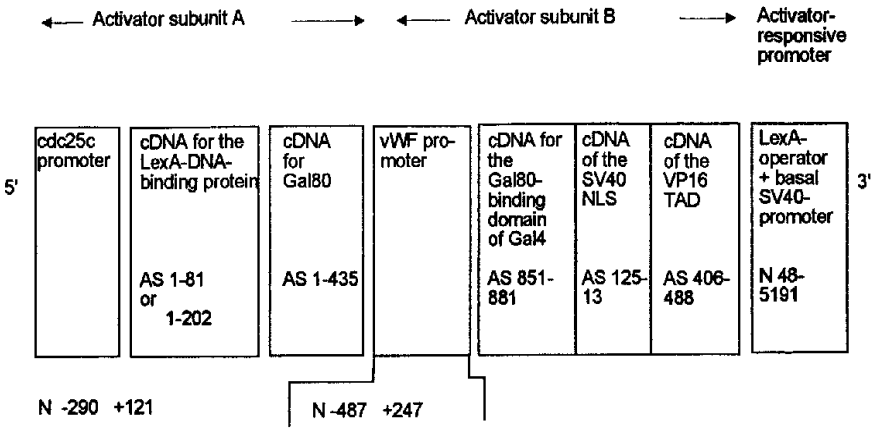


Figure 13

6/8



(N = Nucleotide sequence; AS = Amino acid sequence)

Figure 14

Fusion protein of the activator subunit A

LexA DNA-binding protein	Gal80
- total: AS 1 - 202 or - DNA-binding domain: AS 1 - 81	- total, containing Gal4-binding domain AS 1 - 435

Gal4 - Gal80-binding domain: AS 851 - 881	NLS - SV40 large T: AS 126 - 132	HSV-1 VP-16 trans- activating domain: AS 406 - 488
--	--	--

Fusion protein of the activator subunit B

Figure 15

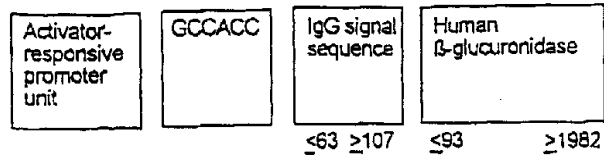


Figure 16

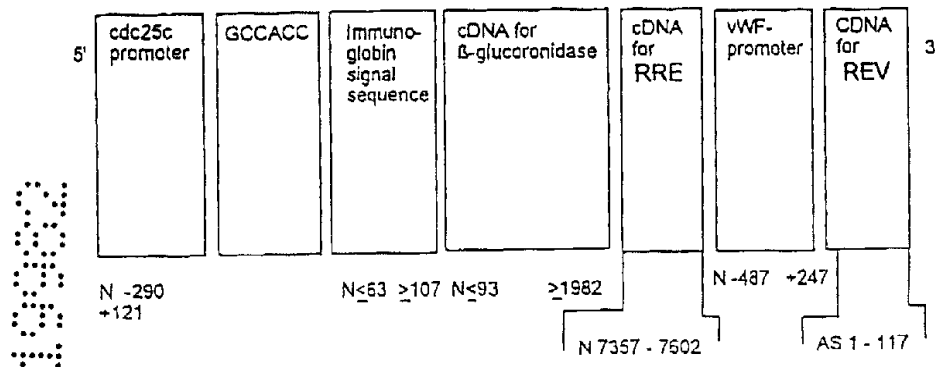
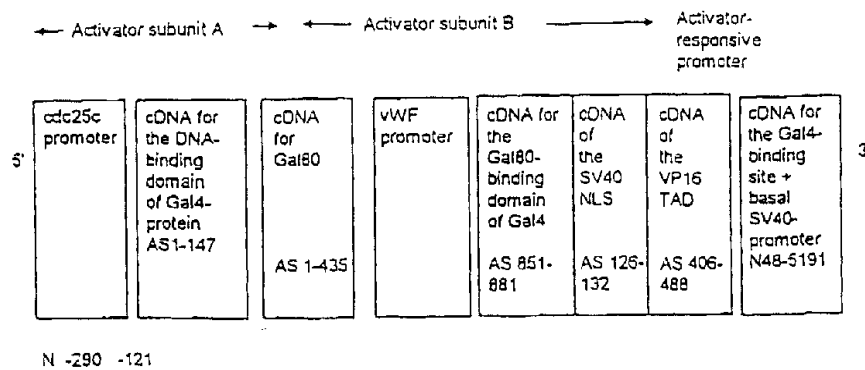


Figure 17



(N = Nucleotide sequence; AS = Amino acid sequence)

Figure 18

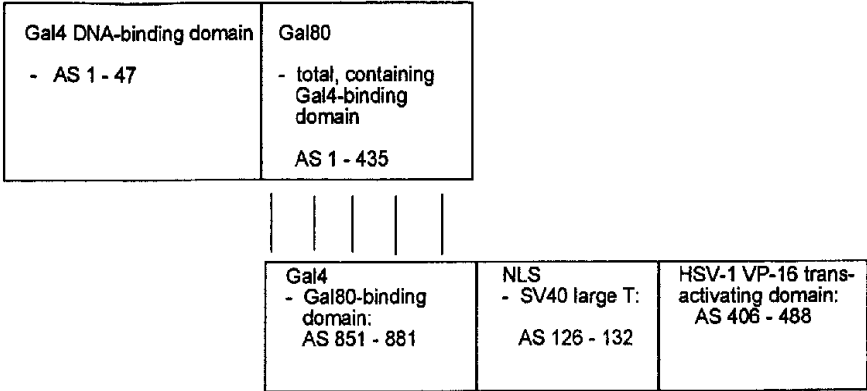


Figure 19

