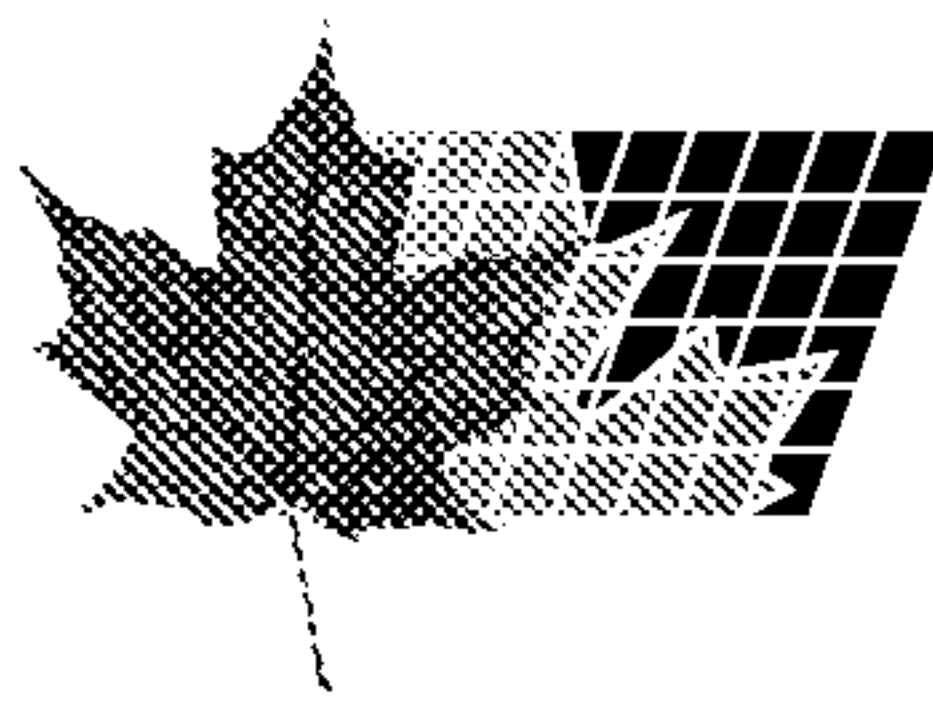




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(51) Int.Cl.⁷ C12N 15/86, A61K 48/00

(30) 1997/08/15 (197 35 593.5) DE

(54) **VECTEUR DE BACULOVIRUS MODIFIÉ PAR COQUE DE
PROTEINE S'UTILISANT EN GENOTHERAPIE**

(54) **COAT-PROTEIN-MODIFIED BACULOVIRUS VECTOR FOR
GENE THERAPY**

(57) L'invention concerne un vecteur de baculovirus modifié par coque de protéine s'utilisant en génothérapie et s'appliquant à la médecine, la biotechnologie et la technique génique. L'invention vise à la construction d'un vecteur de baculovirus présentant une stabilité élevée dans le sang, par modification de l'enveloppe virale et permettant de transférer et d'établir des gènes thérapeutiques, indépendamment de leur taille, dans des cellules mamméliennes, de manière hautement spécifique et efficace. Le vecteur doit pouvoir s'utiliser en génothérapie chez l'homme. Le vecteur obtenu selon l'invention comprend un virus d'insecte, de préférence un représentant des baculovirus ou d'un virus de la polyhédrose nucléaire, contenant les constituants suivants: des coques de protéines de virus modifiées, une séquence d'ADN thérapeutique, un promoteur adapté à l'expression génique et éventuellement une séquence d'établissement.

(57) A coat-protein-modified baculovirus vector for gene therapy is disclosed with applications in medicine, biotechnology and genetic engineering. The object of the invention is the construction of a baculovirus vector with a modified viral coat which makes it highly stable in blood, so that the vector can transfer and establish therapeutic genes of any size with high specificity and effectiveness into mammal cells. The vector should be useful for the gene therapy of humans. The disclosed vector consists of an insect virus, preferably a representative of the baculovirus family or a nuclear polyhedrosis virus, which contains modified virus coat proteins, a therapeutic DNA sequence, a promoter suitable for gene expression, and if required an establishing sequence.

Abstract

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Coat-protein-modified baculovirus vector for gene therapy

5

Description

The invention relates to a coat-protein-modified baculovirus vector for gene therapy, with applications in medicine, biotechnology and genetic engineering.

In recent years, numerous methods and vectors have been developed for gene therapy (summarized in Mulligan/1993/Science 260, 926). Attention has been focused on a large number of vectors, especially ones which have been derived from retroviruses or adenoviruses. Both retroviral and adenoviral vectors have a relatively broad range of applications. Whereas the former is only effective in dividing cells, adenoviruses are also effective in nondividing cells. Although both types of vector are suitable for transferring genes to liver cells (hepatocytes) in vitro, they can hardly be considered for in-vivo gene therapy applications in humans. This is because the use of retroviral vectors necessitates a partial liver excision to stimulate cell division (regeneration), while adenoviral gene transfer is not very stable (no integration in the genome).

Alternative vectors with potential use for hepatocyte gene transfer are based on liposomes or on multi-component particles with protein domains which bind specifically to certain liver receptors (e.g. asialoglycoprotein receptor) and can be incorporated into the cell by means of internalization (overview in: Versland et al/1992/Seminars in Liver Disease 12, 332). A major disadvantage of these vectors is that they enter the cells by way of endocytosis, which leads to disassembly of a large part of the vectors and their DNA in the endosomes. As a result, only a little functional DNA is able to reach the cell nucleus.

A solution to this problem has been found for in-vitro applications, but is not suitable for in-vivo situations (with patients). It is based on the simultaneous infection of the target cells with adenovirus, which leads to disintegration of

the endosomes and release of the vector (DNA) (Curiel, D.T., Agrawal, S., Wagner, E. and Cotten, M. /1991, PNAS 88, 8850-8854).

The DE 44 07 859 suggests a baculovirus vector which is able to transfer therapeutic genes with high specificity and effectiveness into liver cells. Baculoviruses belong to a family of large DNA viruses whose natural host range is restricted exclusively to arthropods. Their genome (80 kbp-200kbp) is packed in flexible nucleocapsids which allow large quantities of foreign DNA to be inserted.

The crucial requirement for baculoviral gene transfer into mammalian cells, and thus also for therapying genetic diseases of the liver, is the insertion of an expression cassette which is able to function in mammalian cells.

The efficiency with which genes are transferred into hepatocytes by baculovirus vectors is reduced, however, by serum components (Sandig et al., 1996; Hum. Gen. Ther. 7:1937-1945). This reduction in efficiency is presumably due to inactivation of the baculovirus vector by complement, and considerably limits therapeutic use of the virus for in-vivo applications.

The object of this invention is the construction of a baculovirus vector which has a modified viral coat to prevent it from being inactivated by serum components and which transfers therapeutic genes with high specificity and effectiveness into liver cells in vivo.

The invention is realized according to claims 1 to 12, the subclaims disclosing preferred variants.

The technologies disclosed in this invention for generating coat-protein-modified baculovirus vectors result in a broad range of novel vectors which, by means of mutation and selection or controlled modification of the baculoviral coat (insertion of receptor ligands), also make it possible to alter the host range and thus to specifically target non-liver cells. In its entirety, the range of baculovirus vectors generated within the scope of this invention is intended to provide a solution for the manifold demands placed on a gene therapy vector, and to be suitable for in-vivo applications in humans.

As therapeutic DNA sequence for the vector of the invention, the cDNA of a gene is used which, in the case of the disease being treated, is defective, i.e. is missing or has undergone change by mutation. It is also possible to use a portion of a genomic sequence that bridges a mutation in the target gene and
5 can recombine homologously therewith.

As promoters, use is made primarily of strong viral promoters, preferably the very early cytomegalovirus (CMV) promoter. Cell-type-specific promoters are also suitable.

10 The function of the establishing sequence is to stabilize the vector in the cell without integration into the genome. It is used especially in cases where long-term expression is required. Preferred establishing sequences according to the invention are viral nuclear establishing sequences such as that of the Epstein-Barr virus, or autonomous replication sequences from the mammal-
15 ian genome.

The new vectors are produced by way of the following main steps:

- I. Mutagenesis and selection method (claim 2, example 1):
 - 20 - Mutagenizing baculoviruses with bromodeoxyuridine
 - Incubating the mutagenized virus with serum
 - Isolating individual virus clones by plaque assay on insect cells
 - Screening for serum resistance and infection spectrum.
- 25 II. Modified coat-protein insertion method (claims 3-6, example 2):
 - Cloning the sequence of an N-terminal-modified baculovirus coat protein (gp64) under the control of a baculoviral promoter in a recombination vector
 - a)
 - 30 - Integrating the construct into the recombination vector which contains the therapeutic DNA sequence together with the promoter
 - If necessary inserting an establishing sequence before or after cloning
 - Transfecting the construct obtained, together with the DNA of an insect virus, into insect cells and

- Recovering the vector packed in the insect cells from the supernatant of the insect cell culture.
- b)
 - Stably integrating the construct into the viral packaging cell
 - 5 - Recovering the coat-protein-modified baculovirus vector by replication of an unmodified vector with a therapeutic expression cassette in insect cells.

10 With this invention, a new kind of gene transfer vector has been developed, which has considerable advantages over viral vectors developed so far (retroviruses, adenoviruses and non-modified baculoviruses). These advantages include stability in blood and serum, liver specificity or freely variable cell targeting, almost unlimited potential for incorporating foreign DNA, infection of nondividing cells, absence of cytotoxicity, and simple generation of high-titer
15 recombinant viruses.

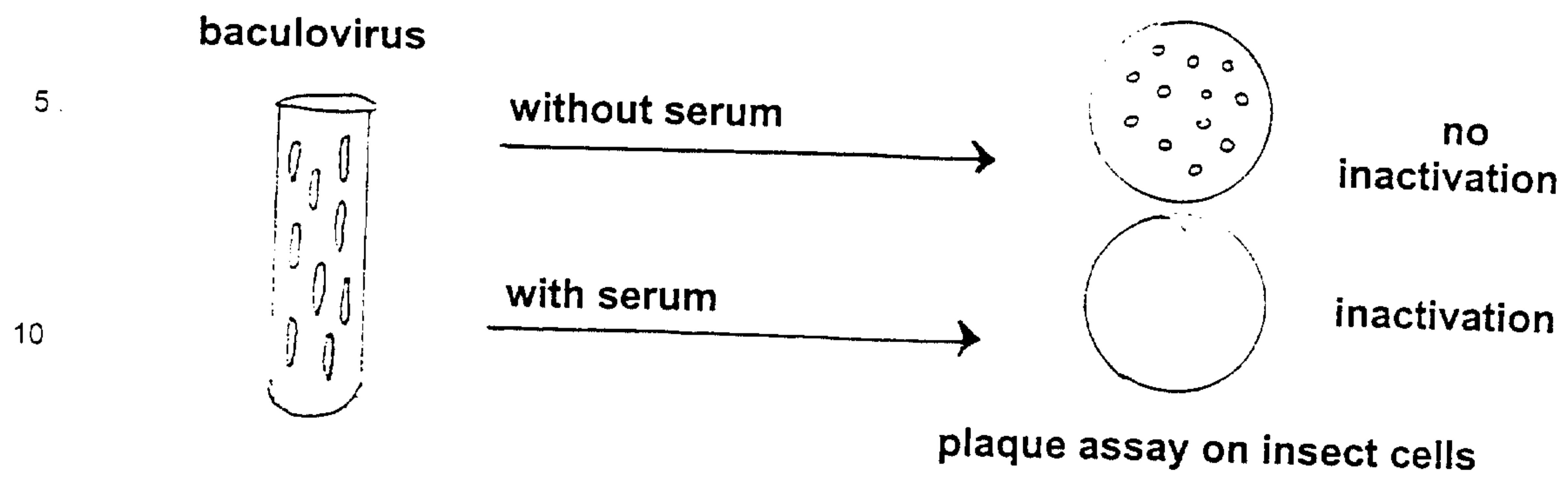
Depending on the type of modification, the coat-protein-modified baculovirus vectors, which can be administered locally or systemically, provide a means of introducing a desired gene into the affected organ of a patient, and of optimizing the pathway to the site where the gene is to function. A primary requirement for the successful therapy of genetic and malignant diseases in
20 humans is thus satisfied.

The invention is now described in detail on the basis of different examples.
Example 1:

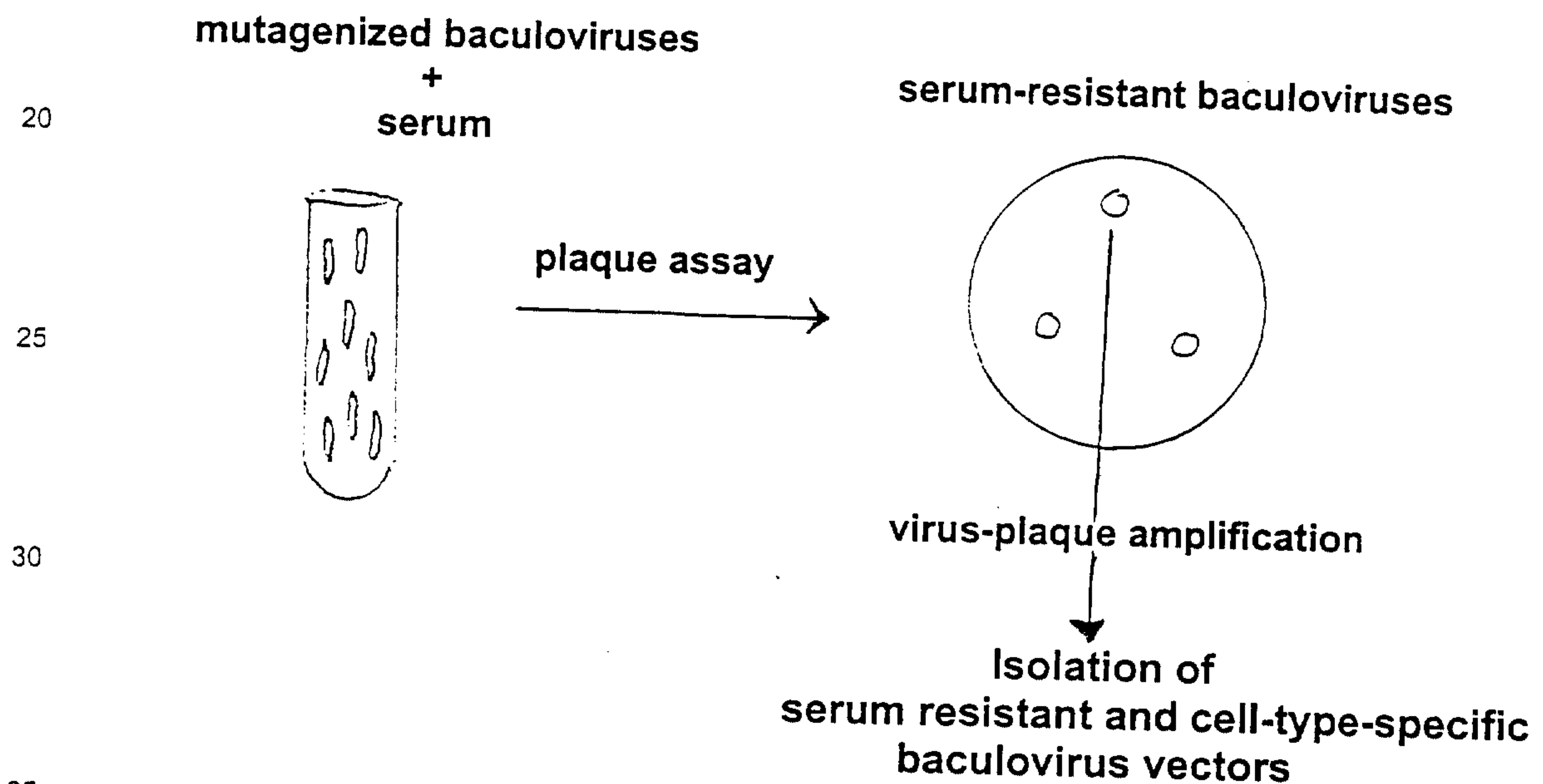
25 I. Mutagenesis and selection method

Gene transfer by baculoviruses is reduced by serum (prior art)
New baculovirus vectors were produced by mutagenizing baculoviruses (10^9 pfu) with bromodeoxyuridine (50 $\mu\text{g/ml}$) in insect cells. 10 μl of the viruses were incubated with 90 μl of serum for 30 minutes at 37 °C. Individual virus
30 clones were isolated using standard techniques by means of plaque assay on insect cells. Individual virus plaques were tested for their ability to infect liver cells following renewed serum treatment. The most effective virus was given the name Baculo-Ser^f. Viruses with a changed host range were also isolated.

PRIOR ART



NEW BACULOVIRUS VECTORS



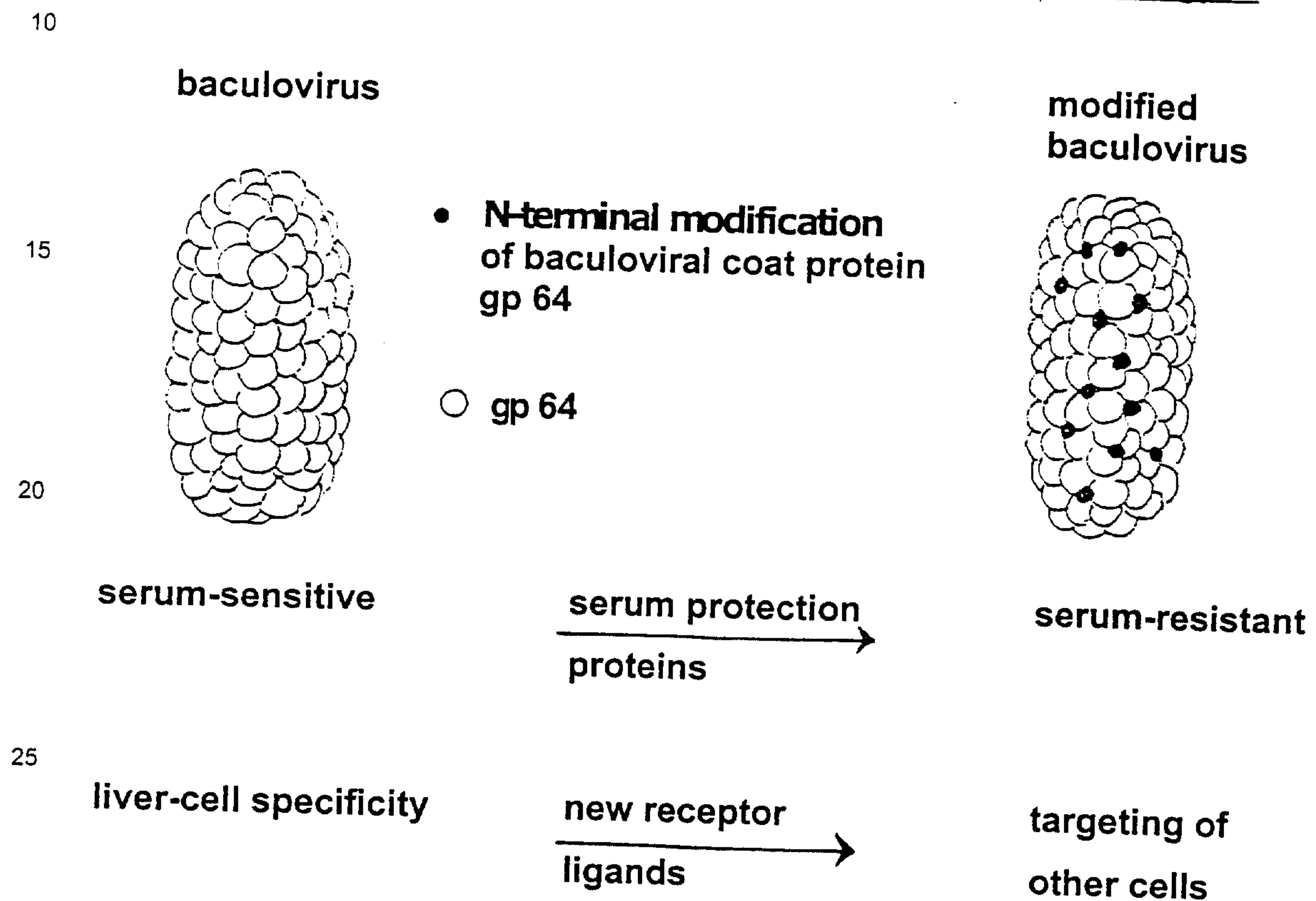
Example 2:

A sequence for an N-terminal-modified baculovirus coat protein (gp64) is cloned – under the control of a baculoviral promoter – in a recombination vector using standard techniques. The modification is accomplished by means of inserting the DNA sequence for amino acids 1-320 of complement protection protein DAF (decay accelerating factor) between the signal sequence and the baculovirus coat protein sequence gp64 at DNA level. This construct is either cloned in the recombination vector, which contains the therapeutic DNA sequences and the promoter, or integrated stably into the

virus packaging cell. For production of the virus, the modified coat protein is inserted into the coat of the baculovirus vector and thereby confers:

- serum resistance by insertion of complement protection proteins or glycosyl transferases according to claims 3 and 5,
- 5 - cell-type-specific infection – other than the liver-cell specificity already demonstrated – by insertion of specific receptor ligands according to claim 4.

GENETIC MODIFICATION OF BACULOVIRUS COAT PROTEIN gp64



New claims

1. A vector consisting of a baculovirus which contains the components
 - modified viral coat proteins
 - a heterologous DNA sequence
 - a promoter for expression in mammalian cells.
2. The vector of claim 1,
wherein
the vector contains an establishing sequence.
3. The vector of claim 1 or 2,
wherein
a therapeutic sequence is used as heterologous DNA sequence.
4. The vector according to one of claims 1 to 3,
wherein
modification of the viral coat proteins is accomplished by mutation and selection for serum resistance and/or an altered host range.
5. The vector according to one of claims 1 to 3,
wherein
modification of the viral coat proteins is accomplished by specific insertion of complement protection proteins, preferably DAF (decay accelerating factor, CD55), MCP (membrane cofactor protein, CD46), CD59 (protectin), or combinations of these.
6. The vector according to one of claims 1 to 3,
wherein
modification of the coat proteins is accomplished by a specific receptor ligand, preferably heregulin, steel factor, CD4 or transferrin.
7. The vector according to one of claims 1 to 3,
wherein

modification of the viral coat proteins is accomplished by altering the glycosylation pattern, preferably by way of glycosyl transferases.

8. The vector according to one of claims 1 to 7,
wherein
the viral coat proteins are modified by DNA sequences incorporated appropriately into the viral genome, or by the stable incorporation of the necessary DNA sequences into the viral packaging cell line.
9. The vector according to one of claims 3 to 8,
wherein,
as therapeutic DNA sequence, the cDNA of the gene or genes is used which are defective in the case of the disease being treated.
10. The vector according to one of claims 3 to 8,
wherein,
as therapeutic DNA sequence, the genomic DNA sequence of the gene or genes is used which are defective in the case of the disease being treated.
11. The vector according to one of claims 3 to 8,
wherein,
as therapeutic DNA sequence, a portion of a genomic sequence is used which bridges a mutation in the target gene and corrects the gene defect by means of homologous recombination.
12. The vector according to one of claims 1 to 11,
wherein
a strong viral promoter or a cell-type-specific promoter is used to promote expression in mammalian cells.
13. The vector according to one of claims 2 to 12,
wherein
a viral nuclear establishing sequence – such as that of the Epstein-Barr

virus – or an autonomous replication sequence is used as establishing sequence.

14. A method of producing the vector disclosed in one of claims 1 to 13,
wherein
the baculoviral coat proteins are modified by
- (a) mutation and selection for serum resistance and/or an altered host range or by insertion of the DNA sequences needed for modification into
 - (b) the baculoviral genome or
 - (c) into the vector-producing insect cell, with the heterologous DNA sequence, together with the promoter, being inserted into the baculoviral genome and the vector being recovered from the supernatant of the insect cell culture.
15. The method of claim 14,
wherein
the heterologous DNA sequence is inserted into the baculoviral genome together with an establishing sequence.
16. A pharmaceutical composition,
wherein
said composition contains a baculovirus as disclosed in one of claims 1 to 13.
17. Use of a recombinant baculovirus vector of the type disclosed in one of claims 1 to 13, or of a pharmaceutical composition according to claim 16, for the production of a gene therapy agent.
18. Use according to claim 17,
wherein
the vector is prepared and administered systemically or locally to a patient in order to therapy the genetic disorder.