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D E C I S I O N
of 6 March 2001

Case Number: T 0239/95 - 3.3.4

Application Number: 83401476.3

Publication Number: 0101655

IPC: C12N 15/00

Language of the proceedings: EN

Title of invention:

Production of herpes simplex viral proteins

Patentee:

AMERICAN CYANAMID COMPANY

Opponent:

SmithKline Beecham Biologicals SA

Headword:

Herpes simplex viral proteins/AMERICAN CYANAMID CO.

Relevant legal provisions:

EPC Art. 84, 83, 123(2), 56

Keyword:

"Main request and auxiliary requests 1 to 3: added subject-matter (yes)"

"Auxiliary request 4: sufficiency of disclosure (yes)"

"Inventive step (yes)"

Decisions cited:

T 0060/89, T 0939/92

Catchword:

-



Case Number: T 0239/95 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 6 March 2001

Appellant: SmithKline Beecham Biologicals SA
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Representative: Dalton, Marcus Jonathan William
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Respondent: AMERICAN CYANAMID COMPANY
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Decision under appeal: Interlocutory decision of the Opposition Division
of the European Patent Office posted 31 January
1995 concerning maintenance of European patent
No. 0 101 655 in amended form.

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: R. E. Gramaglia
S. C. Perryman

Summary of Facts and Submissions

I. The appeal by the appellant (opponent) lies against the decision of the opposition division maintaining European patent No. 0 101 655 (application No. 83 401 476.3) in amended form.

II. The patent with the title "Production of Herpes Simplex viral proteins" was granted on the basis of 20 claims for all designated Contracting States except AT and 19 claims for AT. The relevant claims as granted for all designated Contracting States except AT are as follows:

"1. A DNA sequence coding for a polypeptide comprising the amino acid sequence of a Herpes Simplex virus type 1 or type 2 gD glycoprotein wherein the DNA sequence for the type 1 gD glycoprotein polypeptide comprises:

GGG GGG ACT GCC GCC AGG TTG GGG GCC GTG ATT
TTG TTT GTC GTC ATA GTG GGC CTC CAT GGG GTC
CGC GGC AAA TAT GCC TTG GCG GAT GCC TCT CTC
AAG ATG GCC GAC CCC AAT CGC TTT CGC GGC AAA
GAC CTT CCG GTC CTG GAC CAG CTG ACC GAC CCT
CCG GGG GTC CGG CGC GTG TAC CAC ATC CAG GCG
GGC CTA CCG GAC CCG TTC CAG CCC CCC AGC CTC
CCG ATC ACG GTT TAC TAC GCC GTG TTG GAG CGC
GCC TGC CGC AGC GTG CTC CTA AAC GCA CCG TCG
GAG GCG CCC CAG ATT GTC CGC GGG GCC TCC GAA
GAC GTC CGG AAA CAA CCC TAC AAC CTG ACC ATC
GCT TGG TTT CGG ATG GGA GGC AAC TGT GCT ATC
CCC ATC ACG GTC ACG GAG TAC ACC GAA TGC TCC
TAC AAC AAG TCT CTG GGG GCC TGT CCC ATC CGA
ACG CAG CCC CGC TGG AAC TAC TAT GAC AGC TTC
AGC GCC GTC AGC GAG GAT AAC CTG GGG TTC CTG
ATG CAC GCC CCC GCG TTT GAG ACC GCC GGC ACG

TAC CTG CGG CTC GTG AAG ATA AAC GAC TGG ACG
GAG ATT ACA CAG TTT ATC CTG GAG CAC CGA GCC
AAG GGC TCC TGT AAG TAC GCC CTC CCG CTG CGC
ATC CCC CCG TCA GCC TGC CTC TCC CCC CAG GCC
TAC CAG CAG GGG GTG ACG GTG GAC AGC ATC GGG
ATG CTG CCC CGC TTC ATC CCC GAG AAC CAG CGC
ACC GTC GCC GTA TAC AGC TTG AAG ATC GCC GGG
TGG CAC GGG CCC AAG GCC CCA TAC ACG AGC ACC
CTG CTG CCC CCG GAG CTG TCC GAG ACC CCC AAC
GCC ACG CAG CCA GAA CTC GCC CCG GAA GAC CCC
GAG GAT TCG GCC CTC TTG GAG GAC CCC GTG GGG
ACG GTG GCG CCG CAA ATC CCA CCA AAC TGG CAC
ATC CCG TCG ATC CAG GAC GCC GCG ACG CCT TAC
CAT CCC CCG GCC ACC CCG AAC AAC ATG GGC CTG
ATC GCC GGC GCG GTC GGC GGC AGT CTC CTG GCA
GCC CTG GTC ATT TGC GGA ATT GTG TAC TGG ATG
CAC CGC CGC ACT CGG AAA GCC CCA AAG CGC ATA
CGC CTC CCC CAC ATC CGG GAA GAC GAC CAG CCG
TCC TCG CAC CAG CCC TTG TTT TAC

and the DNA sequence for type 2 gD glycoprotein polypeptide comprises:

ATG GGG CGT TTG ACC TCC GGC GTC GGG ACG GCG GCC
CTG CTA GTT GTC GCG GTG GGA CTC CGC GTC GTC TGC
GCC AAA TAC GCC TTA GCA GAC CCC TCG CTT AAG ATG
GCC GAT CCC AAT CGA TTT CGC GGG AAG AAC CTT CCG
GTT TTG GAC CAG CTG ACC GAC CCC CCC GGG GTG AAG
CGT GTT TAC CAC ATT CAG CCG AGC CTG GAG GAC CCG
TTC CAG CCC CCC AGC ATC CCG ATC ACT GTG TAC TAC
GCA GTG CTG GAA CGT GCC TGC CGC AGC GTG CTC CTA
CAT GCC CCA TCG GAG GCC CCC CAG ATC GTG CGC GGG
GCT TCG GAC GAG GCC CGA AAG CAC ACG TAC AAC CTG
ACC ATC GCC TGG TAT CGC ATG GGA GAC AAT TGC GCT
ATC CCC ATC ACG GTT ATG GAA TAC ACC GAG TGC CCC

TAC AAC AAG TCG TTG GGG GTC TGC CCC ATC CGA ACG
CAG CCC CGC TGG AGC TAC TAT GAC AGC TTT AGC GCC
GTC AGC GAG GAT AAC CTG GGA TTC CTG ATG CAC GCC
CCC GCC TTC GAG ACC GCG GGT ACG TAC CTG CGG CTA
GTG AAG ATA AAC GAC TGG ACG GAG ATC ACA CAA TTT
ATC CTG GAG CAC CGG GCC CGC GCC TCC TGC AAG TAC
GCT CTC CCC CTG CGC ATC CCC CCG GCA GCG TGC CTC
ACC TCG AAG GCC TAC CAA CAG GGC GTG ACG GTC GAC
AGC ATC GGG ATG TTA CCC CGC TTT ATC CCC GAA AAC
CAG CGC ACC GTC GCC CTA TAC AGC TTA AAA ATC GCC
GGG TGG CAC GGC CCC AAG CCC CCG TAC ACC AGC ACC
CTG CTG CCG CCG GAC CTG TCC GAC ACC ACC AAC GCC
ACG CAA CCC GAA CTC GTT CCG GAA GAC CCC GAG GAC
TCG GCC CTC TTA GAG GAT CCC GCC GGG ACG GTG TCT
TCG CAG ATC CCC CCA AAC TGG CAC ATC CCG TCG ATC
CAG GAC GTC GCG CCG CAC CAC GCC CCC GCC GCC CCC
AGC AAC CCG GGC CTG ATC ATC GGC GCG CTG GCC GGC
AGT ACC CTG GCG GCG CTG GTC ATC GGC GGT ATT GCG
TTT TGG GTA CGC CGC CGC GCT CAG ATG GCC CCC AAG
CGC CTA CGT CTC CCC CAC ATC CGG GAT GAC GAC GCG
CCC CCC TCG CAC CAG CCA TTG TTT TAC

or a subsequence of type 1 or type 2 DNA sequence,
which subsequence codes on expression for a polypeptide
having at least one immunological and antigenic
determinant of a Herpes Simplex virus gD glycoprotein."

Claims 2 to 6 were claims to a recombinant vector
comprising the sequences or subsequences according to
claim 1. Claim 7 is a claim to specific recombinant
vectors carried by various deposited microorganisms.

Claims 10 to 11 were claims to unicelleular organisms
containing the sequences or subsequences according to
claim 1.

Claim 12 was a claim directed to various deposited bacteria.

Claim 13 was directed to a process for producing a microcellular organism having a DNA sequence corresponding to the sequences or subsequences according to claim 1.

Claim 14 was directed to a nonglycosylated polypeptide of Herpes Simplex virus type 1 or type 2 gD glycoprotein as listed or a subsequence of the type 1 or type 2gD polypeptide having at least one immunological and antigenic determinant of a Herpes Simplex virus glycoprotein.

Claim 15 was directed to a nonglycosylated polypeptide of Herpes Simplex virus gD glycoprotein produced by a deposited strain according to Claim 12.

Claims 17 and 18 were directed to processes for producing the nonglycosylated polypeptides of claim 14.

Claims 19 and 20 read:

"19. A process for identifying and/or isolating a DNA sequence that codes on expression for a polypeptide having at least one immunological and antigenic determinant of a Herpes Simplex virus gD glycoprotein, which comprises carrying out hybridisation on the DNA sequence under investigation using, as a hybridisation probe, a DNA sequence or subsequence according to claim 1, or a fragment thereof, or mRNA or cDNA derivable therefrom, and identifying and/or isolating those DNA sequences that hybridise with the probe."

"20. A DNA sequence that hybridizes with a DNA sequence or subsequence as claimed in claim 1, or a fragment thereof, and that codes on expression for a polypeptide having at least one immunological and antigenic determinant of a Herpes Simplex virus gD glycoprotein."

Claims 1 to 19 for the Contracting State AT were drafted as corresponding process claims.

III. The Opposition Division came to the conclusion that the patent with a somewhat amended set of claims restricted to HSVgD type 1 put forward as main request at the end of the oral proceedings before it fulfilled the requirements of the EPC.

IV. The following documents are referred to in the present decision:

- (A1) Benett A.M. et al., J. Gen. Virology Vol. 73, pages 2963-2967 (1992);
- (A2) Ludwig H. et al., The Herpes Virus, B. Roizmann Editions, Vol. 2, pages 385-428 (1983);
- (A3) Watson R.J. et al., Science, Vol. 218, pages 381-384 (1982);
- (A4) Weis J.H. et al., Nature, Vol. 302, pages 72-74 (1983);
- (A5) Lee, G. T-Y. et al., J. of Virology, Vol. 43, pages 41-49 (1982);
- (A8) Wagner M.J. et al., Proc. Natl. Acad. Sci. USA, Vol. 78, No. 3, pages 1441-1445 (1981);

- (A9) Villa-Komaroff L. et al., Proc. Natl. Acad. Sci. USA, Vol. 75, No. 8, pages 3727-3731 (1978);
- (A25) Rose J.K. et al., Proc. Natl. Acad. Sci. USA, Vol. 78, No. 11, pages 6670-6674 (1981);
- (EC) Declaration of Dr R.J Eisenberg and Dr G.H. Cohen dated 13 February 1996 (filed by the respondent);
- (EC14) McGeoch D.J. et al., J. Mol. Biol., Vol. 181, pages 1-13 (1985);
- (D30) Declaration of Prof. R.W. Old dated 27 May 1993 submitted in the matter of EP-B-182,442 (Biogen HBV patent, appeal case T 0296/93);
- (D33) Declaration of Prof. W. Fiers dated 3 October 1993 (filed by the appellant).

V. The board issued a communication pursuant to Article 11(2) of the Rules of Procedure of the Boards of Appeal expressing its provisional opinion. The respondent filed on 6 February 2001 a new main request, of which claims 1, 7, 12, 19 and 20 for all designated Contracting States except AT read as follows (the amendments over the corresponding granted claims are shown in bold and by way of deletions):

"1. DNA sequence coding **in substantially isolated form operably linked to a promoter such that the DNA sequence codes on expression** for a polypeptide comprising the amino acid sequence of a Herpes Simplex virus type 1 ~~or type 2~~ gD glycoprotein wherein the DNA sequence for type 1 gD glycoprotein polypeptide

comprises:

GGG GGG ACT GCC GCC AGG TTG GGG GCC GTG ATT
TTG TTT GTC GTC ATA GTG GGC CTC CAT GGG GTC
CGC GGC AAA TAT GCC TTG GCG GAT GCC TCT CTC
AAG ATG GCC GAC CCC AAT CGC TTT CGC GGC AAA
GAC CTT CCG GTC CTG GAC CAG CTG ACC GAC CCT
CCG GGG GTC CGG CGC GTG TAC CAC ATC CAG GCG
GGC CTA CCG GAC CCG TTC CAG CCC CCC AGC CTC
CCG ATC ACG GTT TAC TAC GCC GTG TTG GAG CGC
GCC TGC CGC AGC GTG CTC CTA AAC GCA CCG TCG
GAG GCG CCC CAG ATT GTC CGC GGG GCC TCC GAA
GAC GTC CGG AAA CAA CCC TAC AAC CTG ACC ATC
GCT TGG TTT CGG ATG GGA GGC AAC TGT GCT ATC
CCC ATC ACG GTC ACG GAG TAC ACC GAA TGC TCC
TAC AAC AAG TCT CTG GGG GCC TGT CCC ATC CGA
ACG CAG CCC CGC TGG AAC TAC TAT GAC AGC TTC
AGC GCC GTC AGC GAG GAT AAC CTG GGG TTC CTG
ATG CAC GCC CCC GCG TTT GAG ACC GCC GGC ACG
TAC CTG CGG CTC GTG AAG ATA AAC GAC TGG ACG
GAG ATT ACA CAG TTT ATC CTG GAG CAC CGA GCC
AAG GGC TCC TGT AAG TAC GCC CTC CCG CTG CGC
ATC CCC CCG TCA GCC TGC CTC TCC CCC CAG GCC
TAC CAG CAG GGG GTG ACG GTG GAC AGC ATC GGG
ATG CTG CCC CGC TTC ATC CCC GAG AAC CAG CGC
ACC GTC GCC GTA TAC AGC TTG AAG ATC GCC GGG
TGG CAC GGG CCC AAG GCC CCA TAC ACG AGC ACC
CTG CTG CCC CCG GAG CTG TCC GAG ACC CCC AAC
GCC ACG CAG CCA GAA CTC GCC CCG GAA GAC CCC
GAG GAT TCG GCC CTC TTG GAG GAC CCC GTG GGG
ACG GTG GCG CCG CAA ATC CCA CCA AAC TGG CAC
ATC CCG TCG ATC CAG GAC GCC GCG ACG CCT TAC
CAT CCC CCG GCC ACC CCG AAC AAC ATG GGC CTG
ATC GCC GGC GCG GTC GGC GGC AGT CTC CTG GCA
GCC CTG GTC ATT TGC GGA ATT GTG TAC TGG ATG

CAC CGC CGC ACT CGG AAA GCC CCA AAG CGC ATA
CGC CTC CCC CAC ATC CGG GAA GAC GAC CAG CCG
TCC TCG CAC CAG CCC TTG TTT TAC

~~and the DNA sequence for type 2 gD glycoprotein
polypeptide comprises:~~

~~ATG GGG CGT TTG ACC TCC GGC GTC GGG ACG GCG GCC
CTG CTA GTT GTC GCG GTG GGA CTC CGC GTC GTC TGC
GCC AAA TAC GCC TTA GCA GAC CCC TCG CTT AAG ATG
GCC GAT CCC AAT CGA TTT CGC GGG AAG AAC CTT CCG
GTT TTG GAC CAG CTG ACC GAC CCC CCC GGG GTG AAG
CGT GTT TAC CAC ATT CAG CCG AGC CTG GAG GAC CCG
TTC CAG CCC CCC AGC ATC CCG ATC ACT GTG TAC TAC
GCA GTG CTG GAA CGT GCC TGC CGC AGC GTG CTC CTA
CAT GCC CCA TCG GAG GCC CCC CAG ATC GTG CGC GGG
GCT TCG GAC GAG GCC CGA AAG CAC ACG TAC AAC CTG
ACC ATC GCC TGG TAT CGC ATG GGA GAC AAT TGC GCT
ATC CCC ATC ACG GTT ATG GAA TAC ACC GAG TGC CCC
TAC AAC AAG TCG TTG GGG GTC TGC CCC ATC CGA ACG
CAG CCC CGC TGG AGC TAC TAT GAC AGC TTT AGC GCC
GTC AGC GAG GAT AAC CTG GGA TTC CTG ATG CAC GCC
CCC GCC TTC GAG ACC GCG GGT ACG TAC CTG CGG CTA
GTG AAG ATA AAC GAC TGG ACG GAG ATC ACA CAA TTT
ATC CTG GAG CAC CGG GCC CGC GCC TCC TGC AAG TAC
GCT CTC CCC CTG CGC ATC CCC CCG GCA GCG TGC CTC
ACC TCG AAG GCC TAC CAA CAG GGC GTG ACG GTC GAC
AGC ATC GGG ATG TTA CCC CGC TTT ATC CCC GAA AAC
CAG CGC ACC GTC GCC CTA TAC AGC TTA AAA ATC GCC
GGG TGG CAC GGC CCC AAG CCC CCG TAC ACC AGC ACC
CTG CTG CCG CCG GAC CTG TCC GAC ACC ACC AAC GCC
ACG CAA CCC GAA CTC GTT CCG GAA GAC CCC GAG GAC
TCG GCC CTC TTA GAG GAT CCC GCC GGG ACG GTG TCT
TCG CAG ATC CCC CCA AAC TGG CAC ATC CCG TCG ATC
CAG GAC GTC GCG CCG CAC CAC GCC CCC GCC GCC CCC~~

~~AGC AAC CCG GGC CTG ATC ATC GGC GCG CTG GCC GGC
AGT ACC CTG GCG GCG CTG GTC ATC GGC GGT ATT GCG
TTT TGG GTA CGC CGC CGC GCT CAG ATG GCC CCC AAG
CGC CTA CGT CTC CCC CAC ATC CGG GAT GAC GAC GCG
CCC CCC TCG CAC CAG CCA TTG TTT TAC~~

or a subsequence of type 1 or type 2 DNA sequence, which subsequence codes on expression for a polypeptide having at least one immunological and antigenic determinant of a Herpes Simplex virus gD glycoprotein, with the proviso that the promoter is not the native promoter of Herpes Simplex virus gD glycoprotein.

7. A recombinant vector selected from the group consisting of the recombinant vector pEH51 which is carried by the Escherichia coli having ATCC accession No. 39,159, recombinant vector pEH82 which is carried by the Escherichia coli having ATCC accession No. 39,160, recombinant vector pEH4-2 which is carried by the Escherichia coli having NRRL accession No. B-15471, ~~recombinant vector pHV5 which is carried by the Escherichia coli having NRRL accession No. B-15449,~~ ~~recombinant vector pHV6 which is carried by the Escherichia coli having NRRL accession No. B-15450~~ and recombinant vector pEH90-10am which is carried by the Escherichia coli having NRRL accession No. B-15451.

12. An Escherichia coli bacterium selected from the group consisting of Escherichia coli having ATCC accession No. 39,159, Escherichia coli having ATCC accession No. 39,160, ~~Escherichia coli having NRRL accession No. B-15449,~~ ~~Escherichia coli having NRRL accession No. B-15450~~ Escherichia coli having NRRL accession No. B-15451 and Escherichia coli having NRRL accession No. B-15471.

19. A process for identifying and/or isolating a DNA sequence that codes on expression for a polypeptide having at least one immunological and antigenic determinant of a Herpes Simplex virus gD glycoprotein, which comprises carrying out hybridisation on the DNA sequence under investigation using, as hybridisation probe, a DNA sequence or subsequence according to claim 1, or fragment thereof, ~~or mRNA~~ or cDNA derivable therefrom, and identifying and/or isolating those DNA sequences that hybridise with the probe.

20. A DNA sequence **molecule in substantially isolated form comprising**

(i) a first DNA sequence obtainable from a type 1 or type 2 Herpes Simplex virus, which first sequence that hybridizes with a DNA sequence or subsequence as claimed in claim 1, or with a fragment thereof, and which first DNA sequence ~~and that~~ codes on expression for a polypeptide having at least one immunological and antigenic determinant of a Herpes Simplex virus gD glycoprotein, **and**

(ii) a promoter,

the first DNA sequence as defined in (i) being operably linked to the promoter, with the proviso that the promoter is not the native promoter of Herpes Simplex virus gD glycoprotein."

VI. During oral proceedings held on 6 March 2001, the respondent submitted auxiliary claim requests 1 to 4 (the amendments over the corresponding granted claims are shown in bold and by way of deletions):

Auxiliary request 1

Claims 1 to 18 as in the main request filed on 6 February 2001; claims 19 and 20 as follows:

19. A process for identifying and/or isolating a DNA sequence **obtainable from a type 1 or type 2 Herpes Simplex virus, which sequence** that codes on expression for a polypeptide having at least one immunological and antigenic determinant of a Herpes Simplex virus gD glycoprotein, which comprises ~~carrying out hybridisation on the DNA sequence under investigation using, as hybridisation a probe, a DNA sequence or subsequence according to claim 1, or fragment thereof, or mRNA or cDNA derivable therefrom, and identifying and/or isolating those DNA sequences that hybridise with the probe, and testing the product of those sequences for the presence of an immunological and antigenic determinant.~~

20. A DNA ~~sequence~~ **molecule in substantially isolated form comprising**

(i) **a first DNA sequence obtainable from a type 1 or type 2 Herpes Simplex virus, wherein the sequence as defined in claim 1 can be used as a probe to identify the first DNA sequence as containing gD sequences that hybridizes with a DNA sequence or subsequence as claimed in claim 1, or a fragment thereof, and which first DNA sequence** and that codes on expression for a polypeptide having at least one immunological and antigenic determinant of a Herpes Simplex virus gD glycoprotein, **and**

(ii) a promoter,

the first DNA sequence as defined in (i) being operably linked to the promoter, with the proviso that the promoter is not the native promoter of Herpes Simplex virus gD glycoprotein."

Auxiliary request 2

Claims 1 to 18 as in the main request filed on 6 February 2001; claims 19 and 20 as follows:

19. A process for identifying and/or isolating a DNA sequence **obtainable from a type 1 or type 2 Herpes Simplex virus, which sequence** that codes on expression for a polypeptide having at least one immunological and antigenic determinant of a Herpes Simplex virus gD glycoprotein, which comprises ~~carrying out hybridisation on the DNA sequence under investigation using, as hybridisation a probe, the coding a DNA sequence shown in Figure 3 or subsequence according to claim 1, or fragment thereof, or mRNA or cDNA derivable therefrom, and identifying and/or isolating those DNA sequences that hybridise with the probe, and testing the product of those sequences for the presence of an immunological and antigenic determinant.~~

20. A DNA sequence **molecule in substantially isolated form comprising**

(i) a first DNA sequence obtainable from a type 1 or type 2 Herpes Simplex virus, wherein the coding sequence as shown in Figure 3 can be used as a probe to identify the first DNA sequence as containing gD

~~sequences that hybridizes with a DNA sequence or subsequence as claimed in claim 1, or a fragment thereof, and which first DNA sequence~~ and that codes on expression for a polypeptide having at least one immunological and antigenic determinant of a Herpes Simplex virus gD glycoprotein, and

(ii) a promoter,

the first DNA sequence as defined in (i) being operably linked to the promoter, with the proviso that the promoter is not the native promoter of Herpes Simplex virus gD glycoprotein."

Auxiliary request 3

Claims 1 to 18 as in the main request filed on 6 February 2001; claim 20 deleted; claim 19 as follows:

19. A process for identifying and/or isolating a DNA sequence **obtainable from a type 1 or type 2 Herpes Simplex virus, which sequence** that codes on expression for a polypeptide having at least one immunological and antigenic determinant of a Herpes Simplex virus gD glycoprotein, which comprises ~~carrying out hybridisation on the DNA sequence under investigation using, as hybridisation a probe, a DNA sequence or subsequence according to claim 1, or fragment thereof, or mRNA or cDNA derivable therefrom, and identifying and/or isolating those DNA sequences that hybridise with the probe, and testing the product of these sequences for the presence of an immunological and antigenic determinant.~~

Auxiliary request 4

Claims 1 to 6, 8 to 11, 13 to 18 as in the main request filed on 6 February 2001; claims 19 and 20 deleted; claims 7 and 12 as follows:

7. A recombinant vector selected from the group consisting of the recombinant vector pEH51 which is carried by the Escherichia coli having ATCC accession No. 39,159 **and** recombinant vector pEH82 which is carried by the Escherichia coli having ATCC accession No. 39,160 ~~recombinant vector ppEH4-2 which is carried by the Escherichia coli having NRRL accession No. B-15471, recombinant vector pHV5 which is carried by the Escherichia coli having NRRL accession No. B-15449, recombinant vector pHV6 which is carried by the Escherichia coli having NRRL accession No. B-15450 and recombinant vector pEH90-10am which is carried by the Escherichia coli having NRRL accession No. B-15451.~~

12. An Escherichia coli bacterium selected from the group consisting of Escherichia coli having ATCC accession No. 39,159 **and** Escherichia coli having ATCC accession No. 39,160. ~~Escherichia coli having NRRL accession No. B-15449, Escherichia coli having NRRL accession No. B-15450 Escherichia coli having NRRL accession No. B-15451 and Escherichia coli having NRRL accession No. B-15471.~~

VII. The submissions by the appellant can be summarized as follows:

Main request

Article 123(2) EPC

Claims 1, 19 and 20

- a) There was no basis in the application as filed for the expressions "codes on expression for a polypeptide having at least one immunological and antigenic determinant of a Herpes Simplex virus gD glycoprotein" and for the expression "a DNA sequence in substantially isolated form" (i.e. not within a recombinant vector) in claims 1 and 20 of this request.

- b) There was no specific disclosure in the application as filed for the expression "using, as hybridisation probe, a DNA sequence or subsequence according to claim 1, or fragment thereof, or cDNA derivable therefrom" and "that hybridize with the probe" in claim 19 and "hybridizes with a DNA sequence or subsequence as claimed in claim 1, or with a fragment thereof" in claim 20.

Article 84 EPC

Claims 19 and 20

- c) The expression in claims 19 and 20 "cDNA derivable therefrom" lacked clarity since a cDNA could only be derived from a mRNA upon reverse transcription, not from a DNA.

- d) Claims 19 and 20 lacked clarity because the hybridization conditions were not defined.

Auxiliary request 1

Articles 84 and 123 (2) EPC

Claims 1, 19 and 20

- a1) The same objections as under the main request were raised (see Section VI a)).

- b1) There was no specific disclosure in the application as filed for the expressions "using, as a probe, a DNA sequence according to claim 1, or cDNA derivable therefrom" and "that hybridize with the probe" in claim 19 and "wherein the sequence as defined in claim 1 can be used as a probe" in claim 20.

Auxiliary request 2

Articles 84 and 123 (2) EPC

Claims 1, 19 and 20

- a2) The same objections as under the main request were raised (see Section VI a)).

- b2) There was no specific disclosure in the application as filed for the expressions "using, as a probe, the coding sequence shown in Figure 3 or cDNA derivable therefrom" and "that hybridize with the probe" in claim 19 and "wherein the coding sequence as shown in Figure 3 can be used as a probe" in claim 20.

Article 84 EPC

- c2) The same objections as under the main request were raised (see Section VI c) and d)).

Auxiliary request 3

Article 123(2) EPC

Claims 1 and 19

- a3) The same objections as under the main request were raised (see Section VI a)).

- b3) There was no specific disclosure in the application as filed for the expression in claim 19 "using, as a probe, a DNA sequence according to claim 1, or cDNA derivable therefrom" and "that hybridize with the probe".

Article 84 EPC

Claims 1 and 19

- c3) The same objections as under the main request were raised ((see Section VI c) and d)).

Auxiliary request 4

Article 83 EPC

- Since the respondent argued in the context of the inventive step that at the priority date of the patent in suit the skilled person had to exercise inventive skill to overcome all the problems that arose when attempting to express fusion proteins comprising only portions of gD related polypeptides, the same had to be true when he/she was faced with establishing whether or not a given DNA sequence would have satisfied the requirements of antigenicity and immunogenicity stated in claim 1 or with expressing full-length fused or unfused gD proteins or glycosylated products.

- It would be an undue burden for the skilled person to determine whether any DNA subsequence covered by claim 1 coded on expression for a

polypeptide having at least one immunological and antigenic determinant of a Herpes Simplex virus gD glycoprotein. The immunological reagents available to the skilled person at the priority date of the patent in suit were a few monoclonal antibodies directed against the gD-1 glycoprotein and one polyclonal against HSV-1: these antibodies did not cover the entire range of antigenic determinants susceptible of being expressed in the light of claim 1.

- The patent in suit was merely concerned with the expression in *E. coli* of fusion proteins comprising only portions of gD related polypeptides (cro-gD-like fusion proteins or cro-gD-like β -galactosidase "sandwich" fusion proteins). There was no disclosure in the patent in suit as to how full-length fused, unfused or glycosylated gD proteins could be obtained. As regards expression in *E. coli* of unfused full-length gD protein, document (A25) reported that the signal sequence for a vesicular stomatitis virus (VSV) glycoprotein was toxic to *E. coli*.

Inventive step

- Document (A5) represented the closest prior art because it located the gD gene within a 2.8 kb SacI-SacI DNA fragment, which was used to produce plasmid pRB309. The SacI-SacI DNA insert was hybridized selectively to a mRNA which was shown in vitro to produce a gD polypeptide.

- The claimed subject-matter was within the reach of the skilled person following any of routes A to C below:
 - A. Sequencing the 2.8 kb long SacI-SacI DNA fragment of document (A5). Sequencing DNAs, even with high GC content was within the competence of the skilled person in 1982 (see documents (A6), (A7) and (A8)). Having obtained sequence information to locate the gD coding region precisely, a number of expression strategies were available to the skilled person for inserting portions coding for gD into a suitable expression system.
 - B. Dissecting the SacI-SacI DNA fragment of document (A5) with restriction endonucleases and inserting them into the PstI site of pBR322 as done in document (A14) for Hepatitis B virus (HBV) DNA (shotgun cloning of DNA fragments of HBV into the β -lactamase gene of pBR322).
 - C. Obtaining cDNAs or fragments thereof encoding gD from mRNAs of HSV infected cells (see document (A5)) and proceeding as done in document (A9) for the proinsulin gene, namely the cDNAs are inserted in the β -lactamase gene of pBR322 as in Route B, in the expectation to obtain fusion proteins. This route avoided possible problems due to introns.

VIII. The submissions and evidence provided by the respondent can be summarized as follows:

Main request

Article 123(2) EPC

Claims 1, 19 and 20

- Chapters 5.2 to 5.4 on pages 19 to 25 of the application as filed dealt with expression vectors comprising DNAs encoding HSV gD glycoprotein and the identification of the gene products. This represented a basis for the expressions "which codes upon expression for a polypeptide having at least one immunological and antigenic determinant of a Herpes Simplex virus gD glycoprotein".

- There was also a basis in the application as filed for the expressions in claims 1 and 20 "a DNA sequence in substantially isolated form" (i.e. not within a recombinant vector) on page 16, lines 25 to 29, page 19, line 32 ("Once isolated, the gD gene") and Example 6 on page 33, lines 26 ("the gD-1 gene fragment was isolated") of the application as filed.

- The passage on page 18, line 18 to page 19, line 6 in the application as filed taught in generic terms the use of mRNA or cDNA as a probe to identify by hybridization those fragments containing gD DNA sequences. Moreover, the passage on page 10, lines 2 to 7 taught that HSV gD-1 and HSV gD-2 shared common epitopes. This fact of necessity implied that HSV gD-1 DNA had to hybridize with HSV gD-2 DNA.

- Therefore, the expressions: "using, as hybridisation probe, a DNA sequence or subsequence according to claim 1, or fragment

thereof, or cDNA derivable therefrom" and "that hybridize with the probe" in claim 19 and "hybridizes with a DNA sequence or subsequence as claimed in claim 1, or with a fragment thereof" in claim 20 were directly and unambiguously derivable or at least implied by the text in the application as filed.

Auxiliary request 1

Article 123(2) EPC

Claims 1, 19 and 20

- The same submissions were made as in relation to the main request for the basis in the application as filed for the expressions: "using, as a probe, a DNA sequence according to claim 1, or cDNA derivable therefrom" in claim 19 and "wherein the sequence as defined in claim 1 can be used as a probe" in claim 20 of Auxiliary request 1;

Auxiliary request 2

Article 123(2) EPC

Claims 1, 19 and 20

- A DNA probe could be produced using the information from Figure 3 representing the nucleotide sequence of the gD-1 gene stated in claim 1 and the predicted amino acid sequence of the protein. In Chapter 7.2.2 (pages 70 and 71 of the application as filed) information from the Figures was indeed used for making a gD-1 DNA probe to be used for isolating a gD-2 DNA. Therefore, the expressions: "using, as a probe,

the coding sequence shown in Figure 3 or cDNA derivable therefrom" and "that hybridize with the probe" in claim 19 and "wherein the coding sequence as shown in Figure 3 can be used as a probe" in claim 20 were directly and unambiguously derivable or at least implied by the text in the application as filed.

Auxiliary request 3

Article 123(2) EPC

Claims 1 and 19

- The arguments put forward in relation to the main request for the basis in the application as filed for the expressions in claim 19 "using, as a probe, a DNA sequence according to claim 1, or cDNA derivable therefrom" and "that hybridize with the probe" equally applied for these claims in this request.

Auxiliary request 4

Article 83 EPC

- The patent in suit (see pages 10 to 11, Chapter 5.4: "Identification of the gene product" and Section 6.4.1) disclosed in an enabling manner how to determine whether or not a particular DNA sequence coded on expression for a polypeptide having at least one immunological and antigenic determinant of a HSV gD1 glycoprotein. One could use polyclonal antibodies against HSV (see page 25, line 11) to detect the expression products. Immunogenicity of the expressed proteins could be evaluated by determining test animal antisera titres.

- As regards the unfused gD proteins, claim 1 at issue did not require the production of unfused proteins. The patent in suit disclosed how to express full-length, unfused and glycosylated proteins.

Article 56 EPC

- None of Routes A to C rendered the claimed subject-matter obvious, having regard to the following facts:

Route A

- Sequencing a completely unknown 2.8 kb fragment was not routine at the priority date (GC problem). Prof. Fiers (see document (D33)) was too highly qualified to be considered as a man of average skill.
- Identifying the gD coding region was not straightforward.
- Genetic engineering was still in its infancy at the earliest priority date of the patent in suit.
- Dr Old's declaration is diametrically opposed to an earlier affidavit by him (document (D30); Biogen HBV patent, appeal case T 0296/93).

Route B

- None of the clones of document (A14) expressed HBeAg. HBcAg was merely detected by radioimmunoassays but not confirmed. The authors warned that their experiments were not suited to obtaining expression.
- The potential presence of introns would have dissuaded the skilled person.

Route C

- No monoclonal antibodies recognizing an unglycosylated fragment of gD were available. Only one of the seven anti-gD monoclonal antibodies used in (A5) recognized unglycosylated gD.
- The authors of document (A9) already knew the sequence of their gene which was only 329 bp long, compared with 1,182 bp (gD1). They thus had advantages when solving their problem that the skilled person following route C would not have had faced with the problem to be solved here.
- It was not straightforward to synthesise a long cDNA.

IX. The appellant (opponent) requested that the decision under appeal be set aside and that the European patent No. 0 101 655 be revoked.

The respondent (patentee) requested that the decision under appeal be set aside that the patent be maintained on the basis of the claims of the main request filed 6 February 2001 or of auxiliary request 1, 2, 3 or 4, all submitted at the oral proceedings on 6 March 2001.

Reasons for the Decision

1. The appeal is admissible

Main request

Article 84 EPC

Claims 19 and 20

2. The appellant criticizes the expression in claims 19 and 20 "cDNA derivable therefrom", whereby "therefrom" means DNA, as lacking clarity since a cDNA can only be derived from a mRNA upon reverse transcription, not from a DNA. However, while it is true that a cDNA cannot be derived directly from a DNA (but the claims objected to do not state " derived directly"), it can nevertheless derived via several steps from a DNA via the mRNA (DNA -> mRNA -> cDNA). As for the contention that claims 19 and 20 lack clarity because the hybridization conditions are not defined, this objection relates rather to one of insufficiency (Article 83 EPC) than lack of clarity. The technical meaning of the present wording is clear, namely it covers any DNA hybridizing with the probe in the whole range of stringency, from low to high. In any case any lack of clarity has not been introduced by a post-grant amendment. Therefore, the claims of this request cannot be considered as contravening the requirements of

Article 84 EPC.

Article 123(2) EPC

3. The expression in claims 1, 19 and 20 "which codes upon expression for a polypeptide having at least one immunological and antigenic determinant of a Herpes Simplex virus gD glycoprotein" finds a basis on pages 19 to 25 (Chapters 5.2 to 5.4) of the application as filed dealing with expression vectors comprising DNAs encoding HSV gD glycoprotein and the identification of the gene products. There is also a basis in the application as filed for the expressions in claims 1 and 20 "a DNA sequence in substantially isolated form" (i.e. not within a recombinant vector) on page 16, lines 25 to 29, page 19, line 32 ("Once isolated, the gD gene") and Example 6 on page 33, lines 26 ("the gD-1 gene fragment was isolated").

4. The respondent maintains that the passage on page 18, line 18 to page 19, line 6 represents a basis for the wording in claim 19: "using, as hybridisation probe, a DNA sequence or subsequence according to claim 1, or fragment thereof, or cDNA derivable therefrom" and "that hybridize with the probe" and the expression: "hybridizes with a DNA sequence or subsequence as claimed in claim 1, or with a fragment thereof" in claim 20. The board notes that the cited passage relates to three possible techniques "for identifying the specific DNA fragment containing the gD gene". The first technique is the sequencing of the DNA fragments. The second one is based on mRNA selection. Neither of these two techniques provides any basis for the claimed feature in question. The third technique consists of adsorbing gD-specific mRNAs contained in the polysomes

by means of monoclonal antibodies directed against gD, labelling the mRNA or cDNA derived therefrom and using them as probes to identify the HSV DNA fragments containing gD sequences. In the board's judgement, it is only this latter disclosure which might be considered as a basis in the application as filed for the claim wording in question, but this **specific** polysome-based embodiment producing labelled mRNAs or cDNAs cannot represent a basis for the claimed **general** use, as hybridisation probe, of a DNA sequence or subsequence according to claim 1, or fragment thereof, to identify gD-coding sequences. Moreover, while it is true that the fact that HSV gD-1 and HSV gD-2 share common epitopes (page 10, lines 2 to 7 of the application as filed) of necessity implies that HSV gD-1 DNA has to hybridize with HSV gD-2 DNA, this nevertheless is no direct and unambiguous instruction to "using, as hybridisation probe, a DNA sequence or subsequence according to claim 1, or fragment thereof, or cDNA derivable therefrom". The subject-matter of claims 19 and 20 thus offends against Article 123(2) EPC. Accordingly, the main request comprising these claims has to be refused.

Auxiliary request 1

Article 123(2) EPC

5. The claims of this request differ from those of the main request by the expressions: "using, as a probe, a DNA sequence according to claim 1, or cDNA derivable therefrom" in claim 19 and "wherein the sequence as defined in claim 1 can be used as a probe" in claim 20, namely the "DNA subsequence" has been omitted. As for the corresponding claims of the main request, discussed in point 4. above, these claims are still much broader

than the specific polysome-based embodiment which provides the only possible basis for such subject-matter in the application as filed. Thus claims 19 and 20 of the first auxiliary request also offend against Article 123(2) EPC, and the auxiliary request 1 must be refused.

Auxiliary request 2

Article 123(2) EPC

6. The claims of this request differ from those of the main request by the expressions: "using, as a probe, the coding sequence shown in Figure 3 or cDNA derivable therefrom" in claim 19 and "wherein the coding sequence as shown in Figure 3 can be used as a probe" in claim 20. It is argued by the respondent that the application as filed gives instructions to produce a DNA probe using the information from Figure 3 representing the nucleotide sequence of the gD-1 gene stated in claim 1 and the predicted amino acid sequence of the protein because in Chapter 7.2.2 (pages 70 and 71 of the application as filed), information from the Figures is indeed used for making a gD-1 DNA probe to be used for isolating a gD-2 DNA. The board observes, however, that page 70, line 29 of the application as filed refers to Figure 1d and Figure 4, rather than to Figure 3. In any case, there is no instruction anywhere to use the whole sequence of Figure 3 as a hybridization probe, as required by claims 19 and 20. Thus claims 19 and 20 of the auxiliary request 2 also offend against Article 123(2) EPC, and the auxiliary request 2 must also be refused.

Auxiliary request 3

Article 123(2) EPC

7. The claims of this request differ from those of the main request by the expression in claim 19 "using, as a probe, a DNA sequence according to claim 1, or cDNA derivable therefrom". This does not avoid the arguments for claims 19 and 20 having no basis in the application as originally filed, as set out in point 4. above. Thus claims 19 and 20 of auxiliary request 3 also offend against Article 123(2) EPC, and the auxiliary request 3 must also be refused.

Auxiliary request 4

Article 83 EPC (sufficiency of disclosure)

8. The appellant argues that "there is tension between Articles 56 and 83 EPC" because if the respondent argues that it would involve an inventive step to identify gD epitopes, then the subject matter of claim 1 must of necessity be insufficiently disclosed to be carried out by a skilled person, as the latter would have to exert inventive activity when faced with establishing whether or not a given DNA sequence will satisfy the requirements of antigenicity and immunogenicity stated in claim 1 or when faced with expressing full-length fused, or unfused gD proteins or glycosylated products.
9. In the board's judgement, for the purpose of Articles 56 and 83 EPC, the same level of skill is required from the person skilled in the art (see decision T 60/89, OJ EPO 1992, 268) but in two **different** technical situations: whereas for the purpose of evaluating inventive step the skilled person has knowledge of the prior art only, for the purpose of

evaluating sufficiency of disclosure he/she has knowledge of the prior art **and** of the invention as disclosed.

10. Taking thus both the available prior art and the disclosure of the patent into account, the board observes that the appellant argues in the context of the inventive step that a great many immunological tools were available to the skilled person for identifying gD-1 expression product epitopes. These were collection of monoclonal antibodies recognizing the gD-1 protein and polyclonal antibodies against HSV-1 (see paragraph bridging pages 13 and 14, and the second paragraph of page 14 of the submissions received on 7 February 2001). The board agrees to this position, especially as regards the ability of anti-HSV-1 polyclonal antibodies to bind to gD-1 expression products, which is confirmed by the disclosure of the patent in suit. The latter indeed describes in detail how to produce and detect immunological and antigenic gD-1 polypeptides by means of polyclonal antibodies. Section 6.3.2 thereof discloses the expression of a 2.2 kb fragment containing the last 1026 bp of the carboxy-coding terminus of the gD-1 gene. Section 6.3.3 shows that the expression product is a 46 kD protein consisting of 13 amino acid residues of the cro protein and 342 amino acids of the gD-1 protein. This protein can be precipitated with rabbit antiserum against HSV-1 and by four monoclonal antibodies directed against gD of HSV-1 (page 18, lines 18 to 23). Section 6.4.1 shows that a 160 kD fusion protein reacts with rabbit antiserum against HSV-1 (page 19, lines 39 to 49). Section 6.5.4 shows that a fusion protein of 160 kD and 34 kD binds to rabbit antiserum against HSV-1 (page 24, lines 17 to 25). Finally, the expression products can

also be tested in vivo for their antigenic and immunologic properties, as done in Section 6.4.4, wherein fusion proteins are used for immunizing animals whose antisera are tested for their neutralizing properties (page 21, lines 42 to 46). However time-consuming this technique might be, it does not involve undue burden in the sense of the case law of the boards of appeal (cf decision T 923/92, OJ EPO, 564).

11. As for the appellant's contention that the disclosure of the patent in suit is insufficient for the expression of unfused or glycosylated proteins, the rationale for expressing unfused gD is disclosed under Section 5.2 of the patent in suit, which teaches that "the gD gene or portion thereof can be ligated into an expression vector in a specific site in relation to the vector promoter and control elements so that the gD gene sequence is in reading frame with respect to the vector ATG sequence" (page 9, lines 61 to 63). This passage is followed by the statement: **"Alternatively a gD ATG or synthetic ATG may be used"** (emphasis added). This alternative of necessity implies that the gD natural ATG or the synthetic ATG is the starting codon with no host coding sequence preceding this gD ATG or synthetic ATG, a condition for producing unfused gD proteins. No evidence is before the board that the skilled person cannot practise this instruction. As regards the expression of glycosylated proteins, the appellant provided no evidence that expression of the DNAs of claim 1 would not yield glycosylated gD-1 proteins in a suitable host cell.

12. The appellant maintains that expression in E. coli of unfused full-length gD protein including the N-terminal hydrophobic signal sequence is impossible because

document (A25) reports that the hydrophobic signal sequence of a vesicular stomatitis virus (VSV) glycoprotein is toxic to E. coli. However, it has first to be noted that lethality to the host cell by an expressed product is a sign of its being actually expressed. Secondly, it has to be noted that plasmid pEH51 expresses a 46 kD protein lacking all but the first 6 N-terminal amino acid residues of gD-1 (see patent in suit, page 21, lines 27 to 32), despite the hydrophobic portion spanning from Leu⁸ to Leu²⁰ is well present in the expressed protein. This assumption by the appellant is therefore not convincing.

13. In view of the above findings, the board concludes that no case has been made out that the claims of auxiliary request 4 do not satisfy the requirements of Article 83 EPC.

Inventive step

closest prior art

14. The appropriate starting point for an inventive step analysis is represented by the most accurate knowledge at the priority date of the patent in suit about the position of the gD gene in the HSV genome. This is disclosed by document (A5)(see Figure 5 showing a more precise map location of the gD gene compared with previously published mapping attempts of this gene by Marsden et al. and Ruyechan et al.), which document (A5) the parties agree represents the closest prior art and so does the board. This document locates the gD gene within a 2.4 kb HindIII-SacI DNA region of the HSV-1 genome (between 0.911 and 0.924 map units on the HSV genome shown in Figure 5). A 2.8 kb SacI-SacI DNA fragment including this 2.4 kb HindIII-SacI DNA region

is used to produce plasmid pRB309 which is shown to "efficiently" hybridise to a mRNA which upon translation in vitro produces a gD polypeptide, while plasmid pRB123-3 comprising a leftward overlapping BamHI-HindIII DNA fragment fails to do so (see Figure 1 in combination with the r-h column of page 44).

15. In the light of the said knowledge, the underlying technical problem is defined as being the identification and provision of DNA sequences which code on expression for a polypeptide having at least one immunological and antigenic determinant of a HSV type 1 gD glycoprotein, once they are operably linked to a (non native) promoter.
16. The solution is given by the subject-matter of claim 1 comprising a DNA sequence encoding the gD1 gene. In view of Examples 6.3.3 to 6.5.4 of the patent in suit, showing expression of DNAs coding on expression for a polypeptide having at least one immunological and antigenic determinant of a HSV type 1 gD glycoprotein, the board is satisfied that the above problem has been solved.
17. The relevant question in relation to inventive step is whether, starting from the prior art information referred to in point 14 above, and based on other relevant prior art knowledge, the skilled person would have arrived in an obvious manner at the said DNA molecules, and would have reasonably expected so to arrive.

18. The appellant argues that the claimed subject-matter was obvious for the skilled person following routes A to C below:

Route A

This route involves sequencing the 2.8 kb long SacI-SacI DNA fragment of document (A5) and manipulating the resulting sequencing to insert DNA fragments encoding gD into a suitable expression system. It is the appellant's view that sequencing DNAs, even with high GC content was within the competence of the skilled person in 1982 (see documents (A6), (A7) and (A8)). Having obtained sequence information to locate the gD coding region precisely, the skilled person would have confirmed the gD gene by performing standard molecular biological procedures such as mRNA hybridization and translation. Finally, a number of expression strategies were available to the skilled person for inserting portions coding for gD into a suitable expression system.

19. Much emphasis has been placed by the parties on the question of whether or not it was within the reach of the skilled person at the priority date of the patent in suit to sequence DNA fragments with high GC content, such as the 2.8 kb long SacI-SacI DNA fragment of document (A5). Assuming that the answer to this question is yes, in the board's view, the skilled person would have located two open reading frames (ORFs), namely a first ORF in 5' in the 2.8 kb SacI-SacI DNA fragment and a second 3'-truncated ORF. This is confirmed by later document (EC14), showing in Figure 3 (pages 5 to 6), region gD/US6 (see also Section (h) bridging pages 9 and 10). Bearing in mind

that the AAGCTT motif at positions 5741-5747 of Figure 3 (page 5, third line from the bottom) corresponds to the HindIII site of Figure 3 of the patent in suit and by adding 2,400 bases, ie the length of the HindIII-SacI region (see point 14 supra), one arrives at position 8,141 within the 41k/US7 region (page 6), now termed gI. In the board's view, however, the skilled person was faced with the blockage that neither amino acid nor DNA sequence information regarding the gD gene was available to him/her in order to establish an unambiguous correlation between this protein and the two ORFs. Under these circumstances, the fact that the skilled person might have found possible ORFs in this 2.8 kb fragment would not have represented the decisive breakthrough, in the absence of information that this ORF actually encoded the proteins looked for. This view is supported by document (A8), where a similar situation arose. The authors of this document sequenced a 1800-base pair region of plasmid pX1 but could not identify the thymidine kinase (TK) gene of HSV-1 looked for "because the amino acid sequence of the TK protein is not known". They "relied on a variety of other evidence to locate the gene" (see page 1443, l-h column).

20. Turning to the present situation, the "other evidence" could have been the molecular weight (mw) of the gD protein. In connection with this, gD expressed in vitro according to document (A5) exhibited a mw of 51,000 (see page 46, r-h column) while "the various forms of gD immunoprecipitable from infected cell lysates range in apparent molecular weight from 52,000 to 65,000" (page 44, top of l-h column). "Newly synthesised gD made in the presence of tunicamycin [which inhibits N-linked glycosylation but not O-glycosylation] had an

apparent molecular weight of 50,000" while "newly synthesised gD made in the absence of tunicamycin had an apparent molecular weight of 52,000" (page 46, passage bridging l-h and r-h columns). Having calculated the actual mw of the first ORF and found a value of 43,344 for an unglycosylated peptide (see document (EC14, page 10, l-h column, line 13), the skilled person would have been unable to establish an unambiguous correlation between this first ORF and the mw values of 50,000, 51,000 and 52,000 to 65,000. In view of this confusing situation, it cannot be excluded that the next logical step would have been pursuing the sequencing of the 3'-truncated ORF on the DNA fragment adjacent in 3' to the 2.8 kb SacI-SacI insert of plasmid pRB309 (which is a SacI-SacI insert of plasmid pRB308 of 1.55×10^6 daltons (see document (A5), Figure 1)), in the hope of obtaining an ORF more consistent with these mw values: this would have led the skilled person astray, to arrive in the 41k/US7 region, now termed gI (see document (EC14), Figure 3).

21. A further source of confusion arises from the fact that the 2.8 kb SacI-SacI insert hybridizes to mRNAs encoding other proteins, besides gD. On page 45, r-h column, there are listed other polypeptides encoded by the HSV-1 DNA sequences from the S component and exhibiting mws 68,000, 55,000, 42,000, 33,000 and 22,000. The respondent refers to a 42 kD protein (see point 23 of document (EC)). However, another protein of 55 kD encoded by the 2.8 kb SacI-SacI region can clearly be noted in Figure 5 of document (A5). A comparison of page 44, r-h column: "we conclude that the gD polypeptide is located between **0.911 and 0.924**" with page 47, r-h column: "possibly, the 55,000-molecular-weight polypeptide encoded between **0.911 and**

0.924" (emphasis added) confirms this finding. In view of this overlap of expression products, the skilled person could have reasonably believed that the first ORF 5' in the 2.8 kb SacI-SacI insert of document (A5) encoded for this 55 kD protein or a portion thereof.

22. Finally, the board observes that page 47 (paragraph bridging l-h and r-h columns) of document (A5) teaches that a mRNA encoding the 68 kD polypeptide located in a region designed C' on Figure 1, was spliced. In view of this, the skilled person would not have considered as remote the possibility that the gD gene also contained introns. But if it had been found upon applying route A, also called the "sequencing route", that the coding portion of the gD gene was interrupted by one or more introns, the expression in *E. coli* of fragments of 2.8 kb SacI-SacI region, and so the whole route A, would have been prejudiced because it was known that *E. coli* was incapable of excising introns from the mRNA transcript (see document (D30), point 12). Applying route A seems thus to be only possible with the (ex post facto) knowledge that the gD gene contained no introns: it is doubtful whether the skilled person would have actually adopted the sequencing route without having first solved the problem posed by the potential presence of introns in the gD gene. The board indeed notes that in the patent in suit, priority is given to first solving this problem (see page 17, lines 1 to 2: "The S1 mapping technique demonstrated that both the 3.0 kb and the 1.7 kb mRNA species were unspliced (i.e., did not contain intervening sequences or introns)" and that sequencing the gene occurred only thereafter (see page 17, line 23: "Finally, the HSV-1 of pSC30-4 was sequenced").

23. In view of all these uncertainties and possible traps listed above, the conclusion cannot be drawn that the skilled person had a reasonable expectation of success in adopting route A for identifying and providing DNA sequences which code on expression for a polypeptide having at least one immunological and antigenic determinant of a HSV type 1 gD glycoprotein.

Route B

24. This route consists of digesting the SacI-SacI DNA fragment of document (A5) with restriction endonucleases and randomly inserting the so-obtained subfragments in all possible reading frames into the PstI site of plasmid pBR322. This approach had been adopted by the authors of document (A14) in an attempt to expressing Hepatitis B virus (HBV) DNA. It is a so-called "shotgun cloning" of DNA fragments of HBV encoding HBcAg, HBeAg and HBsAg into the β -lactamase gene of pBR322 in order to obtain by chance fusion proteins. According to the appellant, route B does not require detailed DNA sequence information and provides an expectation of successful expression of β -lactamase-gD fusion polypeptides even if there are introns in the gD coding region.
25. The board observes that of the three possible expressions products disclosed in document (A14), namely β -lactamase-HBcAg, β -lactamase-HBeAg and β -lactamase-HBsAg fusion proteins, only colonies expressing HBcAg epitopes could be identified (see page 45, r-h column), while no HBeAg could be detected (ibidem: "none was producing detectable levels of HBeAg") and as regards HBsAg, only "faint positive reactions were obtained with four clones which are

being analysed further" (see page 46, top of r-h column). It is true that the HBcAg epitope is expressed successfully, however, the appellant does not dispute that this occurs owing to the fortuitous presence of a Shine-Dalgarno sequence 5' to the HBcAg initiation codon rather than under the form of a fusion protein (see page 15 of the submissions dated 6 February 2001). In view of these findings, the board must conclude that the skilled person had no expectation of successful expression of β -lactamase-gD fusion polypeptides by following route B. Moreover, the skilled person could not exclude the presence of introns in the SacI-SacI DNA fragment of document (A5), the occurrence of which would have prevented protein expression in *E. coli* applying route B because of *E. coli*'s incapacity of excising introns (see point 22 supra). This fact further lowered the skilled person's expectation of success following route B.

Route C

26. Route C consists of starting from mRNAs of HSV infected cells (see document (A5), page 42, under the heading "Cells and viruses"), obtaining a pool of cDNAs and screening the cloned cDNAs and isolating those encoding gD with the SacI-SacI DNA fragment of document (A5) as probe, and proceeding as done in document (A9) for the proinsulin gene, namely the gD cDNAs are inserted in the β -lactamase gene of pBR322 as done in Route B, in the expectation of obtaining by chance fusion proteins. In the appellant's view, this route circumvents possible problems due to introns and does not require DNA sequence information.

27. The board notes that the authors of document (A9) had

the advantage of already knowing the only 329 bp long DNA sequence of their gene (rat preproinsulin) because it had already been determined by another group. Therefore, they were able to enrich both the reverse transcript by using a specific primer designed in the light of the known DNA sequence (page 3728, bottom of l-h column) and the cDNA pool encoding preproinsulin by size fractionation (page 3728, top of r-h column). Known DNA sequence information was again relied upon during the screening procedure since they sequenced the screening probe and compared it with the sequence of "Ullrich et al." (see page 3729, l-h column: "We confirmed the presence of insulin DNA in pI19 by direct sequence analysis and screened the rest of the clones with purified pI19 insert labelled by nick translation"). Despite having these advantages, which would not have been available at all to the skilled person faced with applying route C for expressing the 1,182 bp long gene encoding gD1, the authors of document (9) obtained only one clone which produced a fusion protein exhibiting epitopes recognized by anti-insulin antibodies out of the 48 clones identified as containing a cDNA encoding insulin (see page 3729, r-h column). If route C thus only just worked when attempting to identify the rat preproinsulin gene, for which much more identifying information was available than for the present gD type 1 gene, the conclusion cannot be drawn that the skilled person had a reasonable expectation of success in applying route C for arriving at the claimed subject-matter.

28. Furthermore, the board has doubts as to whether this technique could have been practised by the skilled person at all. This is because it is now known that the SacI-SacI DNA fragment of document (A5) also includes

the gI gene region (see point 19 supra) and that three mRNAs of three HSV genes (5, 6 (gD) and 7 (gI); see Figure 2 of document EC(14)) share the same 3' terminus. Therefore, if one used this SacI-SacI DNA as a probe for screening the cloned cDNAs and isolating those encoding gD with the SacI-SacI DNA, as required by the protocol of route C, the number of false positives would have been unacceptably high.

29. In conclusion, the subject-matter of claim 1 cannot be derived in an obvious manner from the prior art. Claims 2 to 6 are directed to recombinant vectors including the the DNA sequence or subsequence of claim 1. Claim 7 is directed to a recombinant vector carried on a deposited E. coli, which vectors are embodiments of Claim 1. Claims 8 to 11 are directed to unicellular organisms containing a sequence according to claim 1. Claim 13 is directed to a process for producing a unicellular organism having a DNA sequence according to Claims 14 and 15 are in effect directed to the nonglycosylated polypeptide encoded by the DNA sequence of claim 1, claim 17 to a process for making the polypeptide of claim 14, and claim 16 to a vaccine comprising such a polypeptide. For any of this claimed subject-matter to be carried out, one must have available the DNA sequences of claim 1. Thus since inventive step can be acknowledged for claim 1, it can be acknowledged for all these other claims as well. This also applies to the claims for the Contracting State AT, drafted as corresponding process.
30. In the judgement of the board no grounds exist under the European Patent Convention which preclude the patent being maintained on the basis of the claims of auxiliary request 4.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.
2. The case is remitted to the first instance with the order to maintain the patent on the basis of the claims of the fourth auxiliary request as submitted at the oral proceedings on 6 March 2001 and a description to be amended.

The Registrar:

The Chairwoman:

P. Cremona

U. M. Kinkeldey