Aktenzeichen / File Number / Numéro du dossier

T0455191 -3.3.2

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- ☐ a copy of the decision correcting errors
- ☐ a corrected covering page (Form 3030)
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- ☐ une copie de la décision rectifiant des erreurs
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- ☐ un sommaire / une phrase vedette (Form 3030)
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Anmeldung Nr. / Patent Nr.: _____ (soweit nicht aus der Anlage ersichtlich)

Application No. / Patent No.: 82300949.3 (if not apparent from enclosure)

Demande n° / Brevet n°: _____ (si le n° n'apparaît pas sur l'annexe)

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0455/91 - 3.3.2

D E C I S I O N
of the Technical Board of Appeal 3.3.2
of 20 June 1994

Appellant: (Proprietor of the patent)
GENENTECH, INC.
460 Point San Bruno Boulevard
South San Francisco
California 94080 (US)

Representative:
Armitage, Ian Michael
MEWBURN ELLIS
2 Cursitor Street
London EC4A 1BQ (GB)

Respondent(s):
(Opponent 01)
Boehringer Mannheim GmbH
Patentabteilung
Sandhoferstrasse 116
D-68298 Mannheim (DE)

Representative:
Huber, Bernhard, Dipl.-Chem.
Patentanwälte
H. Weickmann, Dr K. Fincke
F. A. Weickmann, B. Huber
Dr H. Liska, Dr J. Prechtel, Dr B. Böhm
Postfach 86 08 20
D-81635 München (DE)

(Opponent 02)
Chiron Corporation
4560 Horton Street
Emeryville, California 94608 (US)

(Representative)
Hallybone, Huw George (GB)
c/o Carpmaels & Ransford
43 Bloomsbury Square
GB-London WC1A 2RA (GB)

(Opponent 03)
Celltech Limited
216-222 Bath Road,
Slough
Berkshire SL1 4EN (GB)

(Representative)
-

(Opponent 09) Behringwerke Aktiengsellschaft
Postfach 11 40
D-35001 Marburg (DE)

(Representative) -

(Opponent 10) CIBA-GEIGY AG
Patentabteilung
Postfach
CH-4002 Basel (CH)

(Representative) Zumstein, Fritz, Dr.
Patentanwälte,
Dr. F. Zumstein,
Dipl.-Ing. F. Klingseisen,
Bräuhausstrasse 4
D-80331 München (DE)

Decision under appeal: **Decision of the Opposition Division of the European Patent Office dated 30 April 1991 revoking European patent No. 0 060 057 pursuant to Article 102(1) EPC.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: L. Galligani
E. M. C. Holtz
I. A. Holliday
R. L. J. Schulte

BESCHWERDEKAMMERN DES EUROPÄISCHEN PATENTAMTS | BOARDS OF APPEAL OF THE EUROPEAN PATENT OFFICE | CHAMBRES DE RECOURS DE L'OFFICE EUROPEEN DES BREVETS

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D E C I S I O N
of 20 June 1994

Case Number: T 0455/91 - 3.3.2

Application Number: 82300949.3

Publication Number: 0060057

IPC: C12N 15/00

Language of the proceedings: EN

Title of invention:
Expression of polypeptides in yeast

Patentee:
GENENTECH, INC., et al

Opponent:
(01) Boehringer Mannheim GmbH Patentabteilung
(02) Chiron Corporation
(03) Celltech Limited
(04) ZymoGenetics, Inc.
(05) Delta Biotechnology Limited
(06) Takeda Chemical Industries, Ltd
(07) Novo Industri A/S
(08) Gist-Brocades N.V.
(09) Behringwerke Aktiengesellschaft
(10) CIBA-GEIGY AG Patentabteilung

Headword:
Expression in yeast/GENENTECH

Relevant legal norms:
EPC Art. 54, 56, 123

Keyword:
"Inventive step (no) - obvious to try with reasonable expectation of success"
"Attitude of the skilled person"

Decisions cited:
T 0015/81; T 0195/84; T 0060/89; T 0500/91; T 0223/92

Headnote follows.

BESCHWERDEKAMMERN DES EUROPÄISCHEN PATENTAMTS

BOARDS OF APPEAL OF THE EUROPEAN PATENT OFFICE

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(09) Behringwerke Aktiengesellschaft
(10) CIBA-GEIGY AG Patentabteilung

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Expression in yeast/GENENTECH

Relevant legal norms:
EPC Art. 54, 56, 123

Keyword:
"Inventive step (no) - obvious to try with reasonable expectation of success"
"Attitude of the skilled person"

Decisions cited:
T 0015/81; T 0195/84; T 0060/89; T 0500/91; T 0223/92

Headnote:
A skilled person working in one area of genetic engineering (e.g. expression in yeast) would regard a means found possible in a neighbouring area of genetic engineering (e.g. the bacterial art) as being usable in his own area, if this transfer of technical knowledge appears to be easy and to involve no obvious risks.

Summary of Facts and Submissions

I. European patent No. 60 057 was granted on 6 May 1987 for ten Contracting States with 26 claims and for Austria with 25 claims in response to European application No. 82 300 949.3. The priority of the earlier US application No. 237 913 was claimed (25 February 1981).

II. Notices of opposition were filed against the European patent by ten parties (hereinafter referred to as Respondents I to X) in the period 2 to 5 February 1988.

Revocation of the patent was requested on the grounds of Article 100(a) to (c) EPC. During the procedure before the Opposition Division the parties relied upon a large number of documents [documents (1) to (129)] and declarations. Among them the following are of particular relevance for the purpose of this decision (the numbering used in the decision by the Opposition Division is adhered to):

(5) EP-A-0 001 929;

(10) Proc.Natl.Acad.Sci USA, Vol. 78, No. 4, April 1981, pages 2199 to 2203;

(13A) PhD Thesis of J. L. Bennetzen presented at the University of Washington, 1980;

(24) Abstract No. 112, page 32, 10th International Conference of Yeast Genetics and Molecular Biology, Louvain-La-Neuve, September 8 to 12, 1980;

(28) Cell, Vol. 16, 1979, pages 753 to 761;

(30) Cell, Vol. 20, 1980, pages 215 to 222;

(52) Proc.Natl.Acad.Sci. USA, Vol. 77, No. 1, January 1980, pages 541 to 545;

(61) J. Biol.Chem; Vol. 254, No. 19, 1979, pages 9839 to 9845;

(64) Gene, Vol. 10, 1980, pages 157 to 166;

(120) Science, Vol. 209, 19 September 1980, pages 1428 to 1430;

(122) Yeast Genetics and Molecular Biology 1980, Workshop Reports of the 10th International Conference, Louvain-La-Neuve, September 8 to 12, 1980, pages 51 to 54 (contribution by K. Struhl);

(123) idem as 122, pages 91 to 93 (contribution by M. Guerineau);

(124) idem as 122, pages 95 to 97 (contribution by H. Heslot and P. J. Strijkert).

For the purpose of the present decision the totality of evidence in relation to the oral disclosure of Dr Guarente at 10th International Conference of Yeast Genetics and Molecular Biology, Louvain-La-Neuve, September 8 to 12, 1980 will be referred to as:

document (24').

The said evidence includes document (24), the later publication (document 10), documents (122) to (124) and a number of declarations and affidavits.

III. The Opposition Division, which included also a legally qualified examiner, announced at the end of oral proceedings held on 4 to 5 September 1990 the decision to revoke the patent pursuant to Article 102(1) EPC because both the main request (Claims 1 to 31 for all States except AT and Claims 1 to 30 for AT) and the subsidiary request (Claims 1 to 31 for all States except AT and Claims 1 to 30 for AT) did not meet the requirements of the EPC. The reasoned decision was dispatched on 30 April 1991.

The Opposition Division based its decision substantially on the following arguments:

(a) Claim 9 of both requests did not met the requirements of Article 123(2) and (3) EPC because the use therein of the expression "comprising" in respect of the DNA insert could be interpreted also in the sense of said DNA consisting of three components, namely i) the exogenous DNA, ii) the translation start codon and iii) additional DNA (so-called version B). This aspect of the invention, however, could not be derived from the original application documents.

(b) The subject-matter both of the main and auxiliary request, with the only exception of the embodiments including a transcription termination sequence from a yeast gene, did not involve an inventive step (Article 56 EPC) having regard to the oral disclosure by Dr Guarente at the 10th International Conference of Yeast Genetics and Molecular Biology held in Louvain-La-Neuve on 8 to 12 September 1980 (24') and to the common general knowledge. In fact, the sole difference between the claimed DNA vectors and those of Guarente lay in the fact that the start signal (ATG) was now part of the exogenous DNA insert. However, the skilled person had no difficulties in preparing such an alternative recombinant yeast vector because:

- structural genes encoding a desired polypeptide and preceded by an ATG start signal were known in the art (see document 5);

- the enzymatic tools for annealing, splicing, tailoring DNA fragments were also generally known;

- the expression in yeast of a polypeptide ordinarily exogenous to yeast was also known [see, for example, (24)].

IV. The Appellant (Patentee) lodged an appeal against the decision of the Opposition Division, and submitted its Statement of Grounds together with the declaration of Mr Kim Emmons and further documents designated App. 1 to 8. Two further declarations by Dr J. Corden and Dr R. Zitomer were produced with letter dated 17 December 1993. A letter to the Board by Professor Benjamin Hall and further comments by Professor Michael Smith were filed by the Appellant with letters dated 2 March 1994 and 4 March 1994, respectively.

V. Eight Respondents (Opponents) submitted a response to the appeal.

With their responses Respondents IV/VII and X filed each two additional documents designated, respectively, OP App. 1 & 2 and OX 1 & 2.

With letter dated 27 January 1994, Respondent II filed an affidavit of Professor K. Struhl. Two further affidavits of Professor K. Struhl were filed by Respondent II with letters dated 15 February and 8 March 1994, respectively.

VI. On 17 January 1994 the Board issued a communication pursuant to Article 11(2) of the rules of procedure of the Boards of Appeal with preliminary observations and comments on the case.

VII. The first two days of oral proceedings took place on 15 and 16 March 1994.

During oral proceedings a new main request (Claims 1 to 30) and four new auxiliary requests were filed.

Claim 1 of the main request was as follows:

"A DNA vector suitable for use in expressing exogenous genes in yeast, comprising a sequence which is replicable in yeast, a 5' flanking sequence of a yeast structural gene including its promoter, a site created downstream of said 5' flanking sequence in the direction of transcription for insertion of a structural gene coding for a biocompetent polypeptide ordinarily exogenous to yeast so as to be transcribable under the control of said promoter and translatable from a start signal carried by the DNA insert, and a sequence allowing phenotypic selection of yeast transformants."

Claim 9 of the main request was as follows:

"A recombinant DNA vector for use in expressing an exogenous gene in a suitable yeast strain, comprising a sequence allowing phenotypic selection of yeast transformants, a sequence which is replicable in yeast, a 5' flanking sequence of a yeast structural gene including its promoter which flanking sequence also contains a deletion into the 5' untranslated leader sequence of said gene, and DNA inserted at a site downstream of said 5' flanking sequence so as to be transcribable under the control of said promoter, said DNA insert comprising a sequence encoding a biocompetent polypeptide ordinarily exogenous to yeast and a translational start codon from which said

transcribed coding sequence can be translated in the transformed yeast host."

Claim 28 of the main request was as follows:

"A method of producing a desired heterologous biocompetent polypeptide in yeast by culturing a yeast strain transformed with a recombinant DNA expression vector replicable in said yeast strain, characterised in that the vector contains an inserted exogenous DNA sequence coding for the polypeptide transcriptionally downstream of a 5' flanking sequence of a yeast structural gene containing a promoter which is functional in said yeast strain, which flanking sequence also contains a deletion into the 5' untranslated leader sequence of said gene, and a translation initiation signal other than that endogenous to said yeast structural gene downstream of said promoter and in reading frame with the exogenous coding sequence, so that the exogenous sequence is transcribed from said promoter from said translation initiation signal."

Claims 2 to 8 related to specific embodiments of the vector according to Claim 1. Claims 10 to 19 related to specific embodiments of the recombinant vector according to Claim 9. Claims 20 to 25 and Claims 26 to 27 related to transformed yeast strains transformed with the said recombinant vectors and to methods of forming them, respectively. Claims 29 to 30 related to specific embodiments of the method according to Claims 28.

VIII. At the end of oral proceedings on 16 March 1994, the Board announced that the main request was rejected and adjourned the oral proceedings concerning the auxiliary requests on file until a later date.

IX. By a letter dated 29 April 1994, the Appellant proposed to file sixteen auxiliary claim requests as an attempt to meet the outstanding objections and to arrive at a simple conclusion of the appeal in writing.

In a communication pursuant to Article 11(2) of the Rules of Procedure of the Boards of Appeal the Board informed the Appellant that it did not give its consent to the introduction into the proceedings of the new requests and left to the Appellant's discretion to take into consideration the introduction of the feature "which flanking sequence also contains a deletion into the 5' untranslated leader sequence of said gene" into the auxiliary claims on file.

With letter dated 13 June 1994, the Appellant filed amended auxiliary requests I to III together with a new citation, namely Nature, March 1981, page 77, and the extract from Dr Kingsman's evidence in the High Court of Justice, Chancery Division Patent Court, Biogen vs Medeva. The fourth auxiliary request was withdrawn.

X. Oral proceedings were continued on 20 June 1994.

The Board did not give its consent to the introduction into the proceedings of the auxiliary requests I to III filed with letter dated 13 June 1994. The Board accepted the introduction of the feature "which flanking sequence also contains a deletion into the 5' untranslated leader sequence of said gene" into the auxiliary requests I to III previously on file. Auxiliary requests I to III incorporating the said amendment were distributed at the oral proceedings as the basis of the appeal review.

Auxiliary request I (Claims 1 to 30) corresponds essentially to the main request with the difference

that all claims are formulated as method claims. Moreover, Claim 1 therein contains the feature "which flanking sequence also contains a deletion into the 5' untranslated leader sequence of said gene" which is absent in Claim 1 of the main request.

Claim 1 is identical in auxiliary requests II (Claims 1 to 41) and III (Claims 1 to 40) and reads as follows:

"A DNA vector suitable for use in expressing exogenous genes in yeast, comprising a sequence which is replicable in yeast, a 5' flanking sequence of a yeast structural gene including its promoter which flanking sequence also contains a deletion into the 5' untranslated leader sequence of said gene, a site created downstream of said 5' flanking sequence in the direction of transcription for insertion of a structural gene coding for a biocompetent polypeptide ordinarily exogenous to yeast so as to be transcribable under the control of said promoter and translatable from a start signal carried by the DNA insert, a sequence allowing phenotypic selection of yeast transformants, and a transcription termination sequence provided by a flanking sequence of a yeast gene downstream of said insertion site".

Claim 11 in auxiliary request II reads as follows:

"A recombinant DNA vector for use in expressing an exogenous structural gene in a suitable yeast strain, comprising a sequence allowing phenotypic selection of yeast transformants, a sequence which is replicable in yeast, a 5' flanking sequence of a yeast structural gene including its promoter which flanking sequence also contains a deletion into the 5' untranslated leader sequence of said gene, and DNA inserted at a site downstream of said 5' flanking sequence so as to

be transcribable under the control of said promoter, said DNA insert comprising a sequence encoding a biocompetent polypeptide ordinarily exogenous to yeast and a translational start codon from which said transcribed coding sequence can be translated in the transformed yeast host, and a transcription termination sequence for said DNA downstream of said insertion site."

Claim 11 of auxiliary request III differs from Claim 11 of auxiliary request II in that the transcription termination sequence is further specified as being "provided by a flanking sequence of a yeast gene".

For simplicity's sake no reference is made here to the remaining claims of the said auxiliary requests (for further details, reference is made to the file).

XI. The Appellant argued that for a proper evaluation of the inventive merit of the claimed subject-matter due account had to be taken of the state of the art prior to the present patent which was as follows:

(a) Although some exogenous genes had been expressed in yeast [see documents (6), (8), (74)], it was not possible to draw a definite conclusion on why expression had been achieved. Fortuitous recognition of the foreign promoter by yeast was just one of many possible explanations. Transcriptional readthrough from a distant promoter or fortuitous recognition of another non-promoter sequence were other possibilities.

(b) It was abundantly clear that bacterial expression differed in many important ways from eukaryotic expression, both at transcriptional and translational levels. Among eukaryotes much

less was known about yeast than about mammalian cells. There was no straightforward connection between bacteria and yeasts. In particular, in respect of the promoters and their mechanism of operation, striking differences were observed in the state of the art between bacterial and eukaryotic organisms.

(c) Although some sequence data were available with respect to the 5' and 3' flanking sequences of yeast genes [see (28), (13A)], the boundaries of these regions were not known. There was unclarity about the exact location of the promoter regions of yeast genes, in particular of their 3' boundaries, and about the possible presence of leader regions upstream of the start codon and their possible influence on translation initiation [see, for example, documents (28) and (52)].

(d) The so-called first AUG rule (see declaration by Dr Zitomer) was a working model proposed to account for the selection of the start site during translation which, however, was not concerned with promoter effects during transcription. The observation that translation of mRNA started at the first available AUG did not mean that all that was required for expression was the first AUG. The proposed model did not give account of the phenomena taking place between the initiation of transcription and the initiation of translation.

(e) Document (30) had described experiments in which, by means of heavy random mutation exercises in a mutant yeast gene which lacked the natural start codon, ATG codons were

generated in the region -3 to 9 and expression of the protein was measured. No DNA sequence data were provided to show what the mutation was and where it was. No systematic investigation of the effect of intended changes in the DNA sequence in the translation initiation region was made. This work gave support to the hypothesis that also in yeast translation initiated at the most proximal AUG codon. However, this document did not lead to the conclusion that one could dispense with the native leader sequence and that the start signal could be used out of its natural context. Nor did document (30) allow the conclusion that there were no sequences important for translation initiation upstream of the said start signal.

(f) The disclosure by Dr Guarente (24') showed that the gene encoding the "reporter" molecule lacZ' could be expressed in yeast when attached to two differently sized fragments of the yeast Cyc1 coding region. Different levels of expression and different types of expression regulation were observed. One of the explanations given for these differences was the presence of promoter regulatory elements downstream of the position which were resected in the shorter of the two constructs. This work supported the idea that yeast promoters were indeed large and complex [see also comments by Dr Struhl in document (122)]. Dr Guarente had not tried to resect the 3' terminus of the promoter region. When he made deletions, these were a long way upstream of the mRNA start signal because he - on the basis of the prior art knowledge - could not assume that sequences around the natural ATG

could be deleted or altered. Dr Guarente did not know whether and to what extent the yeast promoters might have involved internal elements or important elements at the boundary of the coding region. The 3' boundary of a yeast promoter had neither been investigated, nor determined, nor mapped.

The Appellant submitted that the present patent had clarified the function of the region at the 3' region of the promoter immediately upstream of the start codon by showing for the first time that one could dispense with the native leader region in yeast and that the said region was free for inserting a heterologous gene of choice coding for a biocompetent polypeptide. The state of the art as discussed above, also in the light of (24'), could not have made this obvious because of the many uncertainties and because no information was available about the 3' boundaries of yeast promoters and, moreover, this region was regarded as being "sacrosanct". Only after the recognition by the present inventors that the DNA sequence immediately upstream of the translation initiation codon was not important for efficient expression, it was possible to provide yeast vector constructs ready for the insertion of a coding sequence of an exogenous biocompetent polypeptide of choice together with a translational start codon.

In respect of the transcription termination feature which further characterized the claimed subject-matter in auxiliary requests II and III, the Appellant argued that only little information was available in the art about the presence of DNA sequences involved in termination in yeast [see, for example documents (28) and (13A)]. No evidence was yet available that these sequences could be functional. Thus, in its submissions, it would not have been obvious for the

skilled person in 1981 to place a discrete termination unit downstream of the gene to be expressed in a yeast vector construct as done in the present patent.

XII. The Respondents considered that by omitting the expression "and other than those required for growth of the transformant" in respect of the biocompetent polypeptide ordinarily exogenous to yeast, the Appellant violated Article 123(2) EPC because the application as originally filed was directed to a polypeptide having the two inseparable features, i.e. "being a biologically competent polypeptide ordinarily exogenous to yeast" and "other than those required for growth of the transformant" (see page 6, last paragraph).

Moreover, objection was raised under Article 123(2) EPC to the inclusion of the expression "which flanking sequence also contains a deletion into the 5' untranslated leader sequence of said gene" in the claims. In their submissions, there was no basis whatsoever in the application as originally filed for such an amendment.

All Respondents objected under Article 123(2) and (3) EPC to the expression "provided by" used in relation to the transcription termination sequence (see, for example, Claim 1 in auxiliary requests II and III). In their submission this constituted an inadmissible form of "intermediate generalisation" with respect to the granted claims. Moreover, no basis for such an expression could be found in the application as originally filed.

Respondent III objected under Article 84 EPC to the product claims because these contained wording which defined the structural features of the product in terms

of the process by which the features were introduced ("created", "carried by", "provided by" et cetera). In its submission this resulted in extreme difficulty in understanding the proper scope of the claims.

Furthermore, all Respondents considered that the claimed subject-matter of all requests did not involve an inventive step essentially for the following reasons:

(1) At the priority date gene expression in bacteria was a very well developed art which was within the capacity of the skilled person. Methods and means therefor were known. Techniques for engineering around the start codon were used routinely (see, for example, documents (120) and (5)]. Document (120), for example, disclosed the tailoring of a bacterial promoter and its fusion to an inserted ATG-gene so as to obtain direct expression.

(2) The similarities between bacterial and yeast expression were recognised in the art [see document (12)]. The skilled person knew in macroscopic terms what was needed for expression in yeast. Moreover, yeast was considered desirable as a possible host (5).

(3) A number of yeast genes had been cloned and sequenced. Upstream and downstream sequences had been identified [see, for example document (28)]. It was recognised that the essential elements of the yeast promoter were the 5' flanking sequences and there was support for the model in which the translation started at the first AUG [see document (28)].

Document (30) had demonstrated that the context of the ATG could be changed through mutation and expression could still be obtained. This demonstrated that there was nothing essential in the region around the ATG and suggested flexibility in the position of the initiation codon.

(4) Dr Guarente had shown that it was possible to obtain heterologous gene expression in yeast under the control of a yeast promoter (24'). His presentation which was concerned both with bacterial and yeast vector constructs comprising the lacZ gene established a direct link between bacterial and yeast expression systems.

The said presentation was made before a group of people who were able to immediately identify the significance thereof as demonstrated by the comment made at the workshop by Dr Heslot (124) that "this system obviously can be used to obtain expression of other foreign genes in yeast".

(5) The disclosure of Dr Guarente (24') combined with the prior art finding [see, for example document (30)] that no exact spacing was needed between the ATG start codon and the promoter for normal expression rendered obvious for a skilled person the preparation of yeast vector constructs for the direct expression of heterologous genes such as those of the present claims. The engineering of the 3' end of the promoter to ensure that the first AUG encountered by the ribosome was the first AUG of the gene to be expressed was entirely obvious from the bacterial art. Thus, the preparation of

said constructs involved nothing else than the application of known techniques in a reasonable expectation of success.

The Appellant neither proved an increase in efficiency of expression nor put forward any surprising, unpredictable results in connection with the claimed constructs. It was clear that claims related to a matter of mere technical convenience for obtaining the known goal of producing expression products which did not have modified N termini. However, this was entirely predictable on the basis of the prior art.

(6) As for the problem of providing a proper transcription termination which underlay auxiliary requests II and III, it was evident for the skilled person that also yeast mRNAs had discrete 3' ends determined by termination sequences. Thus, it was obvious to include termination sequences in a yeast vector construct. The presence of transcription termination sequences in yeast genes was explicitly referred to in the prior art [see e.g. (28) and (13A)]. Thus, the skilled person had every motivation to use them in the construction of yeast expression vectors. In any case, the obviousness of including such sequences had nothing to do with whether or not they would ultimately prove to be important for foreign gene expression. The present patent neither disclosed any specific termination signal nor described any unexpected or surprising effect in relation to their use so that in the end its teaching in this respect was not different from the prior art teachings.

XIII. The Appellant requested that the decision under appeal be set aside and the patent be maintained on the basis of the main request as filed on 16 March 1994 or, alternatively on the basis of the first, second or third auxiliary request as distributed on 20 June 1994.

The Respondents requested that the appeal be dismissed.

Reasons for the Decision

1. The appeal is admissible.

2. *Formal admissibility [Article 123 (2) (3) EPC]*

As a result of the amendments the subject-matter of the claims of all requests is **more narrowly** defined than it was in the claims as granted. Consequently, the extent of protection conferred by the claims is **reduced** in comparison with that conferred by the claims as granted. Thus, the requirements of Article 123 (3) EPC are met.

Also the requirements of Article 123(2) EPC are met because the said amendments find support in the original application documents. In particular:

i) the application as originally filed explicitly referred in general terms to production of a "biocompetent polypeptide" (see page 4, third paragraph). The reference to biocompetent polypeptides "other than those required for growth of the transformant" in the original application documents is to be regarded as a particular embodiment. Thus, in the Board's view, the omission of the latter expression from

the independent claims does not constitute a violation of Article 123(2) EPC.

ii) As for the introduction in the claims of the expression "which flanking sequence also contains a deletion into the 5' untranslated leader sequence of said gene", the Board observes that, although the reference to the leader sequence and to deletions in the said region is found in the original application documents only in connection with the specific example of the alcohol dehydrogenase (ADH) gene, nevertheless it is unambiguously derivable from the whole context that the exemplified teaching is meant to be generally applicable (see, for example, page 24, fourth paragraph and page 25, fourth paragraph). Thus, the amendment cannot be considered to result in subject-matter which extends beyond the content of the application as filed.

iii) As for the use of the expression "provided by" in the auxiliary request II and III, the Board is of the opinion that it finds full support in the application as originally filed since this made clear that transcription terminations were supplied in the form of 3' flanking sequences of yeast genes (see page 18, sixth paragraph and page 25, second paragraph). In original claim 2 the word "provision" is found.

3. *Clarity (Article 84 EPC)*

In the Board's opinion, the clarity requirement of Article 84 EPC is met by all newly filed claims of all requests. The expressions objected to by Respondent III do not leave doubts as to the category and the scope of

the claims. In the whole context of the claims, the said expressions are used to specify the position or the origin of a structural feature. Nor do said features render the claims obscure from a technical point of view.

4. *Novelty (Article 54 EPC)*

4.1 The closest prior art

The oral disclosure by Dr Guarente at the 10th International Conference of Yeast Genetics and Molecular Biology held in Louvain-La-Neuve on September 8 to 12, 1980 (24') represents the closest prior art.

The evidence available indicates that at the poster presentation and during two workshops at the said conference Dr Guarente illustrated his studies on the characterisation of the Cyc1 (iso-1-cytochrome c) promoter and on the use therefor of the lacZ' gene. The aim of these studies was the elucidation of the function of the said promoter. Dr Guarente disclosed the construction of plasmids which contained fusions of the E.coli lacZ' gene to a region flanking Cyc1 to the 5' side and which carried selectable markers and origins of replication for both E.coli and S.cerevisiae, in particular the construction of the plasmid corresponding to pLG669-Z shown in Figure 1 of the later publication (10). He reported that both E.coli and S.cerevisiae cells transformed with the said plasmids were capable of expressing ß-galactosidase under the Cyc1 promoter. The lacZ' gene represented in the said constructs the exogenous DNA sequence.

Plasmid pLG669-Z (plasmid with the inserted exogenous DNA sequence = "loaded vector") - as he disclosed - was

constructed from plasmid pLG669 (plasmid before the insertion of the exogenous DNA sequence = "empty vector") by inserting into the Bam HI site the lacZ' coding sequence. This precursor plasmid pLG669 comprised the following structural elements: **i)** a yeast replicon; **ii)** a 5' flanking sequence of a yeast structural gene including its promoter (and the first four nucleotides **ATG**A of the Cyc1 gene, i.e. the start codon and one additional nucleotide); **iii)** a site downstream thereof for inserting a gene (Bam HI site); **iv)** a yeast marker; **v)** a bacterial marker and **vi)** a bacterial origin of replication.

Dr Guarente described also certain deletions in the said 5' flanking sequence, in particular the deletion of the XhoI(-700)-XhoI(-250) fragment. The latter deletion resulted in the reduction of expression in yeast, but not in E.coli. Thereby the probable location of the Cyc1 promoter region within the 1100 nucleotide region which preceded the CYC1 structural gene in the yeast chromosome was identified. Moreover, the location of the Hogness box (ca. 120 nucleotides upstream of the start of the Cyc1 coding sequence) was also identified.

In the course of the present proceedings there was a general consensus on the overall contents of the oral disclosure by Dr Guarente as depicted above.

4.2 None of the Respondents contested novelty in respect of any of the claims of the new requests vis-à-vis the oral disclosure (24').

It is observed that the gene encoding the lacZ' "reporter" molecule falls under the definition of "a structural gene coding for a biocompetent polypeptide ordinarily exogenous to yeast" in the same meaning as used in the present case.

The vectors according to Claims 1 and 9 as well as the method according to Claim 28 of the main request are novel vis-à-vis the vectors and methods known from (24') because:

- the "empty" vector according to Claim 1 does not contain the ATG codon of the yeast gene which affords the promoter;

- the "loaded" vector of Claims 9 and 28 bears a deletion into the 5' untranslated leader sequence of the yeast gene which affords the promoter.

Thus, no novelty objection applies to the main request.

The independent claims of auxiliary requests I to III all incorporate inter alia the feature of the deletion into the 5' untranslated leader sequence. Thus, there can be no novelty objection also in respect of these requests for the reasons already given.

5. *Inventive step (Article 56 EPC)*

5.1 **Main request**

5.1.1 The technical problem

In the light of (24') the problem to be solved by the patent-in-suit can be seen in the construction of alternative yeast expression vectors suitable for expressing in yeast any exogenous gene of choice.

5.1.2 The solution proposed

As a solution thereto, Claim 1 proposes the construct of an "empty" vector which bears downstream of the 5' flanking sequence of a yeast gene which affords the

promoter a site for insertion of the exogenous DNA sequence of choice with its own start signal. In this "ready-for-use" vector, the start signal of the said yeast gene is missing. Claim 9, on the other hand, proposes a "loaded" vector bearing a deletion into the 5' untranslated leader sequence of the yeast gene which affords the promoter.

The patent specification gives examples of vector constructs in which a DNA sequence encoding leucocyte interferon D is inserted together with its start signal downstream of the 5' flanking sequence of the yeast ADH gene including the promoter and having deletions extending through the native start signal into the 5' untranslated leader region. Yeast cells transformed with such vector constructs are shown to express a biologically active product (see Table 1).

5.1.3 Assessment of inventive step

5.1.3.1 Dr Guarente provided with his oral disclosure (24') what was perceived at the conference as the first demonstration of expression in yeast of a sequence exogenous thereto under the control of a yeast gene promoter [see in this respect in particular the comments in document (124)].

5.1.3.2 As it is the normal task of the skilled person to be constantly occupied with the elimination of deficiencies, with the overcoming of drawbacks and with the achievement of improvements of known devices and/or products (in this respect see, for example, decisions T 15/81 OJ EPO 1982, 2, point 3 of the Reasons and T 195/84, OJ EPO 1986, 121, point 8.1 of the Reasons), it is realistic to assume that a skilled person aware of the presentation by Dr Guarente (24') would have readily considered the problem of providing

alternatives and/or improvements to the disclosed vectors and methods.

5.1.3.3 A number of decisions in the field of biotechnology have already provided a definition of the person skilled in the art for the purpose of Article 56 EPC (see, for example, T 60/89, OJ EPO 1992, 268, see point 2.2.4 of the Reasons; T 500/91 dated 21 October 1992, see point 2.2 of the Reasons and T 223/92 dated 20 July 1993, see point 5.5 of the Reasons, both not published in the OJ EPO).

The Board considers it useful for the purpose of the present decision to make some considerations on what is believed to be the attitude of the said person skilled in the art vis-à-vis possible changes, modifications and/or adjustments in known products (e.g. a plasmid) or procedures (e.g. an experimental protocol). This with a view to provide a possibly objective answer to the question whether or not the introduction of a given change in a structure or in a procedure can be seen as obvious for the skilled person, avoiding any ex post facto analysis.

In the Board's view, the skilled person in this field is well aware of the fact that even a small structural change in a product (e.g. a vector, a protein, a DNA sequence) or in a procedure (e.g. a purification process) can produce dramatic functional changes. Therefore, the said expert would constantly be conditioned by the prior art and, before taking action, would carefully ponder any possible modification, change or adjustment against the background of the existing knowledge. Under these circumstances, in the Board's view, the skilled person would adopt **a conservative attitude**. However, this must not be seen in the sense of being reluctant or opposed to modify or

adjust a known product or process, but rather in the sense of being **cautious**. For example, the skilled person in question would neither go against an established prejudice nor try to enter into "sacrosanct" or unpredictable areas nor take incalculable risks. However, within the normal design procedures, the said expert would readily seek appropriate, manifest changes, modifications or adjustments which involve little trouble or work and no risks or only calculable risks, especially for the sake of obtaining a more handy or convenient product or of simplifying a procedure. In particular, the skilled person working in one field (e.g. expression in yeast) would regard a means conveniently adopted in a neighbouring field (e.g. the bacterial art) as being readily usable also in that field, if this transfer of technical knowledge involves nothing out of the ordinary.

5.1.3.4 Taking the above considerations into account, the proper question to ask is not whether the skilled person could have tried to modify the technical teaching disclosed in document (24'), but whether he or she would have done so. In seeking an answer to this question it should be borne in mind that the skilled person in the field of expression of polypeptides in yeast had good reasons to move in the direction of the technical teaching of the patent-in-suit, because the skilled person knew how to adjust the technical teaching in (24') from an adjacent neighbouring field, namely the bacterial art. This was a sufficient incentive for an expert at least to try to transform knowledge from the bacterial art to yeast. It is observed that in this respect the expert in the bacterial art and for yeast is the same.

5.1.3.5 Turning now to the solution proposed in Claim 1 of the patent-in-suit, the Board observes that the "empty" vector of this claim differs from the "empty" plasmid disclosed in (24') only in that it does not contain the start signal (ATG) of the yeast gene which affords the promoter. The idea beyond this proposal by the present patent is to provide a "ready-for-use" vector in which a DNA sequence consisting of start signal-exogenous DNA sequence-(optionally) termination signal can be inserted. This approach, however, was already known from the bacterial art [see, for example, document (120), in particular figure 1 (A)]. Nothing in the prior art indicated to the skilled person concerned with the construction of yeast expression vectors that the said approach would not have been usable in yeast. Moreover, for the skilled person it would have made no difference whether the start signal was already in place within the vector or it was introduced together with the exogenous DNA sequence, as long as it was properly positioned within the vector.

In the Board's view, the structural change which makes the difference between the vector of Claim 1 and the "empty" plasmid of (24') is one that a skilled person occupied with the construction of alternative yeast expression vectors would have readily considered. The introduction of such a change into the known plasmid vector required for a skilled person nothing out of the ordinary and thus involved no inventive skill, all being a matter of technical convenience.

5.1.3.6 The "loaded" vector of Claims 9 and 28 differs from the "loaded" plasmid disclosed in (24') in that it contains deletions into the 5' untranslated leader sequence of the yeast gene which affords the promoter. A key question with respect to inventive step of these claims is whether a skilled person, starting from the teaching

of (24'), would have readily considered making such deletions.

In this respect the Appellant substantially maintains that the skilled person would not have taken the incalculable risk of carrying out any engineering in the said region because little was known about its possible influence on expression in yeast. Moreover, in its submissions, too little was known about yeast promoters, in particular about their 3' boundaries, to render obvious for the skilled person making deletions in the 5' untranslated leader region. Thus, in the Appellant's opinion, the skilled person regarded this region as "sacrosanct".

The Board observes that in early 1981 a number of yeast genes had been cloned and sequenced and the upstream and downstream sequences therein had been identified [see, for example, document (13A), in particular Figure 25, and document (28)]. Promoter sequences were known to be located in the upper region of the 5' flanking sequences [see documents (13A) and (24')]. The model in which translation started in yeast at the first AUG downstream from the 5' terminus of the mRNA, with no other sequence requirements found wide support and acceptance [see documents (28), (30) and (120)]. In particular, the studies disclosed in document (30) had established that there was no absolute requirement for a particular sequence 5' to the initiation codon and that translation started at the AUG codon closest to the 5' end of the mRNA. The latter document is particularly relevant to the present discussion and deserves further analysis.

5.1.3.7 The studies of document (30) were carried out by performing mutations in the Cyc1 locus of a mutant yeast strain that lacked the normal ATG codon so as to

obtain intragenic revertants in which the initiation codon was relocated within the region occupied by codons -3 to 9. It was observed that normal amounts of iso-1-cytochrome c occurred when translation initiated at the sites corresponding to codon positions -3, -2, 3 and 5 as well as the normal position -1 and it was therefore concluded that the initiation translation codon could be located anywhere within a region spanning 37 nucleotides and presumably at any site preceding and following the site of the normal initiation codon.

The Appellant insists that the studies reported in document (30):

- dealt with mutations, **not** deletions;

- were concerned with the initiation of translation, **not** with transcription;

- did not report any explicit DNA sequence data.

Thus, in its submissions, the said studies did not allow the conclusion that alterations could readily be introduced into the leader sequence of a yeast gene.

The Board is rather of the view that the skilled person would have readily concluded from document (30) that alterations, at least by way of mutation, in the region just upstream of the start signal (leader region) in the DNA were feasible. This is because:

- the changes in the position of the translation initiation codon reported in document (30) with reference to the mRNA sequences reflected the alterations produced by the mutations at the

corresponding site into the DNA. No explicit DNA data were necessary to recognize this;

- the fact that translation had taken place with normal or near-normal efficiencies in the mutated intragenic revertants, must have implied that transcription, which precedes translation in DNA expression, had also occurred rather undisturbed.

Consequently, the Board believes that the skilled person on the basis of the teaching of document (30) would not have regarded the region immediately preceding the start signal of a yeast gene (the 5' untranslated leader region) as being "sacrosanct" or "untouchable" in spite of alleged uncertainties about a possible function of the leader sequence. The fact that the introduction of point mutations in this region had not sensibly changed translation efficiency rather indicated that alterations therein were feasible.

5.1.3.8 It still remains to be established whether a skilled person faced with the problem of providing alternative yeast expression vectors would have considered modifying those known from (24') by carrying out deletions into the 5' untranslated leader sequence of the yeast gene which afforded the promoter.

In respect of this question, it is observed that document (30) expressly indicated that the initiation codon could be located inter alia at a site preceding its normal position (e.g. at the site corresponding to codons -3, -2). This amounted for the skilled person to the teaching that by producing a change of the codons through mutation the start signal could be moved towards the promoter, upstream of its normal position, i.e. into the 5' untranslated leader region. The skilled person knew from the bacterial art [see, for

example, document (120), in particular on page 1429, left hand column, the passage referring to Figure 1 (B)] that the distance between the promoter and the ATG start signal could be varied by way of resection with nucleases. From the said prior art the skilled person knew that the distance between the promoter and the start signal could be optimized. Thus, one of the possible modifications into the "loaded" plasmid of (24') which would have readily occurred to the skilled person was the changing of the distance between the start signal of the exogenous gene and the promoter that was known to be located in the upper region of the 5' flanking sequence of the yeast gene (see point 4.1, fourth paragraph, above). In order to perform such modification the skilled person had two possibilities:

- either produce by mutation a change in the codons preceding the natural start signal as taught by document (30); or

- resect or produce deletions into non-essential regions upstream of the said start signal as known from the bacterial art.

Both possibilities required for the skilled person nothing out of the ordinary in the field and involved only routine trials.

Thus, also in view of the considerations made above in point 5.1.3.3, the Board concludes that it would have been obvious for a skilled person to try with a reasonable expectation of success to modify the "loaded" plasmid of (24') by producing one or more deletions into the 5' untranslated leader sequence of the yeast gene which afforded the promoter. In view of the prior art [especially documents (24') and (30)], the skilled person would have regarded this

modification as feasible and as involving only fully calculable risks, if any. Thereby the skilled person would have readily arrived at a yeast vector according to Claim 9 and at its use for expressing an exogenous polypeptide in yeast according to Claim 28. This was a matter of normal design procedures for which neither "creative thinking" nor "inventive talent" were necessary.

5.1.3.9 For the above reasons, Claims 1, 9 and 28 lack an inventive step and the main request is consequently not allowable.

5.2 **Auxiliary request I**

Claims 1 to 30 of this request are essentially the same as Claims 1 to 30 of the main request with the only difference that all claims are formulated as method claims. Claim 1, however, contains the feature "which flanking sequence also contains a deletion into the 5' untranslated leader sequence of said gene" which is absent in Claim 1 of the main request.

In the Appellant's submission this request represents what **was done**.

In the Board's view, the mere change of product claims into method claims is not enough to confer an inventive step to their subject-matter if the characterising features of the claimed methods are the same which characterise the product. This is precisely the case here. Since it has been concluded above in respect of the main request that the skilled person needed no inventive skill in order to arrive at the claimed "empty" and "loaded" vectors, it must necessarily be concluded that also the methods for preparing them did not require inventive skill. Therefore, auxiliary

request I is not allowable for the same reasons given above in respect of the main request.

5.3 **Auxiliary requests II and III**

5.3.1 The technical problem and its solution

For both of these requests the closest prior art is represented by the oral disclosure of Dr Guarente (24').

The technical problem derivable therefrom is the same as for the main request, namely the construction of alternative yeast expression vectors suitable for expressing in yeast any exogenous gene of choice (see point 5.1.1, above).

As a solution thereto, the two requests provide in Claim 11 the construct of a "loaded" vector and in Claim 1 the construct of an "empty" vector.

As already stated (see section X, above), Claim 1 is identical in both auxiliary requests II and III. Thus, in respect of the "empty" vector the same solution is offered.

Claim 11 differs in the two requests in that the feature related to the presence of "a transcription termination sequence for said DNA downstream of said insertion site" which characterises Claim 11 of auxiliary request II is further specified in Claim 11 of auxiliary request III by the expression "provided by a flanking sequence of a yeast gene".

5.3.2 Assessment of inventive step

Inventive step is discussed here in relation to the solution as represented by the claimed "loaded" vectors of both requests (see Claim 11).

The "loaded" vectors of auxiliary requests II and III differ from the "loaded" vector of the main request (see Claim 9 therein) in that they contain a transcription termination sequence downstream of the inserted exogenous DNA, this sequence being provided by a flanking sequence of a yeast gene in the case of auxiliary request III.

In points 5.1.3.6 to 5.1.3.9 above it was concluded that the "loaded" vector according to claim 9 of the main request did not involve an inventive step. Thus, the relevant question now is whether or not the additional feature of the presence of a termination sequence confers an inventive step to the claimed vectors.

In the Board's view, a "cautious" skilled person occupied - in the light of documents (24') and (30) - with the construction of alternative yeast expression vectors would have given careful consideration to all structural elements believed to be suitable and/or necessary for expression in yeast.

The skilled person knew that sequences at the 3' end of yeast genes beyond the translation stop codon were associated with the termination of RNA transcription [see, for example, document (13A), in particular page 7, Figure 25, page 134, line 10 to 136, line 3, Table V and page 141, last paragraph to page 145, line 13; document (28), in particular Figure 2 and page 759, left hand column, paragraphs 3 and 4;

document (52), in particular page 544, right hand column to page 545, left end column, first paragraph and figures 3 and 4; document (61), in particular figure 2b; document (64), in particular page 164, right hand column, last paragraph].

In the Board's opinion, although not much information on the precise structure and mechanism of action of such yeast termination sequences was available in early 1981, the skilled person occupied with the construction of alternative yeast expression vectors would have readily considered the inclusion therein of a reasonably large, discrete fragment comprising a termination sequence (cf. Claim 11 of auxiliary request II), in particular the inclusion of a fragment consisting of the 3' flanking sequence of a yeast gene (cf. Claim 11 of auxiliary request III), downstream of the exogenous DNA sequence. In view of the information available, the skilled person would have expected this structural measure to ensure proper transcription termination, i.e. the functional role normally exerted in yeast. Nothing in the art indicated that such a measure could have prejudiced expression. On the contrary, based on the cautious experimental approach "when in yeast, do like yeast does", as the Respondents put it, the skilled person would have considered such a structural measure as being appropriate for the purpose of achieving effective expression. As DNA sequences comprising termination sequences were known (see above), the construction of yeast expression vectors containing them would have involved for the skilled person routine experimental work comprising only routine trials.

5.3.3 For the above reasons, Claim 11 of both auxiliary requests II and III lacks an inventive step and the said requests are consequently not allowable.

Under these circumstances, it is not necessary to discuss inventive step in relation to the "empty" vector.

Order

For these reasons it is decided that:

1. The appeal is dismissed.

The Registrar: The Chairwoman:

P. Martorana U. Kinkeldey

Schmitt 2.9.94