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D E C I S I O N
of 27 November 2002

Case Number: T 0627/01 - 3.3.4

Application Number: 83301296.3

Publication Number: 0088632

IPC: C12N 15/62

Language of the proceedings: EN

Title of invention:
Expression, processing and secretion of heterologous protein by yeast

Patentee:
GENENTECH, INC.

Opponents:
Behringwerke Aktiengesellschaft
DSM Gist Holding B.V.
Novo Nordisk A/S
The Green Cross Corporation

Headword:
Secretion of proteins/GENENTECH, INC.

Relevant legal provisions:
EPC Art. 56

Keyword:
"Main request - inventive step - yes"

Decisions cited:
T 0354/97, T 0455/91

Catchword:
-

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Case Number: T 0627/01 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 27 November 2002

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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 10 April 2001 revoking European patent No. 0 088 632 pursuant to Article 102(1) EPC.**

Composition of the Board:

Chairwoman: U. M. Kinkeldey
Members: F. L. Davison-Brunel
S. C. Perryman

Summary of facts and submissions

I. European patent No. 0 088 632 with the title "Expression, processing and secretion of heterologous protein by yeast" was granted with 13 claims for all Designated Contracting States, based on European patent application No. 83 301 269.3.

II. It was opposed by four parties. The decision of the Opposition Division was appealed as appeal case T 354/97 which was decided on 3 May 2000. The then competent Board acknowledged sufficiency of disclosure in relation to, and the novelty of, the fourth auxiliary claim request on file and remitted the case to the Opposition Division for further prosecution on the basis of this request.

Claim 1 read as follows:

"1. A process for obtaining human protein heterologous to a yeast organism as a product of yeast expression, processing and secretion, which process comprises culturing viable yeast cells transformed with an expression vehicle functionally harboring DNA encoding said human protein together with a heterologous signal peptide therefor, said heterologous signal peptide being heterologous to the yeast and not normally being produced or employed by said yeast organism, said culturing resulting in secretion of said human protein into the medium of the culture, and further comprising the step of recovering said human protein from the medium of the culture."

Claims 2 to 9 related to further features of the process of claim 1. Claim 10 related to a yeast expression vehicle harbouring the human DA leukocyte DA signal sequence fused to a DNA encoding human leukocyte A protein.

The corresponding claims were filed for the Designated Contracting State AT.

III. The Opposition Division came to the conclusion that the subject-matter of claim 1 failed to fulfill the requirement of Article 56 EPC and revoked the patent.

IV. The Appellants (Patentees) filed an appeal, submitted the grounds of appeal and paid the appeal fee. The main claim request on appeal for all Contracting States except AT is the request refused by the Opposition Division, the wording "heterologous to a yeast organism" being deleted from claim 1. This wording is retained in the corresponding claim 1 for AT.

V. Respondents I and II (Opponents 2 and 4) are parties as of right to the proceedings. Opponents 1 and 3 withdrew their oppositions before the first appeal took place.

VI. A summons to oral proceedings to take place on 27 November 2002 was issued. With letters dated 4 and 5 November 2002 respectively, the Appellants and Respondents I informed the Board that they would not attend these proceedings.

VII. The documents mentioned in the present decision are the following:

(P3): Hitzeman, R.A. et al., Nature, Vol.293, pages 717 to 722, 1981,

(P4): Talmadge, K. et al., Proc. Natl. Acad. Sci. USA, Vol. 77, No. 6, pages 3369 to 3373, 1980,

(P7): Mercereau-Puijalon, O. et al., Gene, Vol. 11, pages 163 to 167, 1980,

(P19): Perlman, D. et al., Proc. Natl. Acad. Sci. USA, Vol. 79, pages 781 to 785, 1982,

(P20): Talmadge, K. et al., Proc. Natl. Acad. Sci. USA, Vol. 77, No. 7, pages 3988 to 3992, 1980,

(P31): Taniguchi ,T. et al., Proc. Natl. Acad. Sci. USA, Vol. 77, No. 9, pages 5230 to 5233, 1980,

(P50): Talmadge, K. et al., Nature, Vol. 294, pages 176 to 178, 1981,

(P53): Novick, P. et al., Cell, Vol. 25, pages 461 to 469, 1981,

(T-9): Chan, S.J. et al., Proc. Natl. Acad. Sci. USA, Vol. 78, No. 9, pages 5401 to 5405, 1981,

VIII. The submissions in writing by the Appellants may be summarized as follows:

- The closest prior art was document (P3) which described the intracellular expression of mature human leukocyte interferon in yeast.

Starting from this document, the problem to be solved could be defined as to improve or provide an alternative means for the production of a human protein in yeast.

The solution provided was the process according to claim 1 involving the expression of a DNA encoding a signal sequence which was not of yeast origin fused to the coding sequence of the human gene to be expressed, which expression led to the secretion of the protein in the yeast cells culture medium.

- There was no incentive in document (P3) to secrete a protein at all. Thus, to arrive at the subject-matter of claim 1, the person skilled in the art had to conceive of secreting the protein in the first place.

- Had the skilled person thought of this approach, he/she would not have had a reasonable expectation of success of being able to carry it out. This was especially true since document (P3) described the failure to express rat growth hormone in yeast when the growth hormone coding sequence was preceded by its natural (ie. of rat origin) secretion signal sequence. One possible cause for this failure was said to lie within the signal sequence itself.

- As for the Respondents' arguments concerning the alleged universality of the secretion pathway and therefore, the obviousness of using any secretion signal in yeasts, they were not convincing. Indeed, even if as mentioned in document (P3), page 722, 2nd column, the secretion pathway of yeast may have been thought to follow the general pathway used by animal cells, this did not mean that a heterologous signal sequence would direct secretion in yeasts. In the same manner, the fact that secretion signals from genes of higher organisms were sometimes recognized in bacteria did not necessarily imply that they would also be recognized by yeasts, bacteria being much less fastidious than these latter organisms ie. having a much less complicated secretory pathway.

- The facts of the case where the patent was revoked for lack of inventive step in decision T 455/91 (OJ EPO 1995, 684) were clearly different from the present facts. The then claimed yeast construct was an empty plasmid which only differed from the plasmids of the prior art in that it did not contain an ATG at the point of insertion of the foreign coding sequence. Exactly analogous empty vectors were described in the bacterial art and ATG was known to be the universal translation initiation codon. In view of this prior knowledge, the Board, then, found that it would not require anything out of the ordinary from the skilled person to conceive of, and to isolate the claimed vectors.

Here, the only disclosure which involved the utilisation of the secretion signal of a higher organism in yeast, namely the rat experiment in document (P3), would have dissuaded the person skilled in the art from following the secretion route although this route had already been tried, but not always successfully, to produce foreign proteins in E.coli (documents (P4), (P50) and (P31)).

IX. The submissions in writing and during oral proceedings by Respondents II may be summarized as follows:

- The closest prior art was document (P3) which described the expression of mature human leukocyte interferon in yeast. Since the naturally occurring signal sequence was deleted when cloning the gene for expression in yeast, the interferon was produced intracellularly. On page 722, 2nd column, it was stated about yeast: "*... the secretory pathway follows the general pathway used by animal cells*[22]*.*".

Starting from document (P3) the problem to be solved could be seen as providing a process which allowed for the extracellular production in yeast of human polypeptides.

The solution provided in claim 1 was to fuse the coding region of the polypeptide to be expressed to a signal sequence which was heterologous to the yeast host cells and which allowed for secretion into the yeast culture medium.

- The above mentioned statement provided a clear hint that yeast may be used for secretion and furthermore, at the priority date, secreting proteins had already been widely acknowledged as an efficient means for producing them (document (P4)). Thus the proposed solution was obvious to try.

- In addition, the secretory pathway was thought to be universal as pointed out, for example, in documents (T-9) or (P53) and it had already been shown that eukaryotic signal sequences worked in procaryotes (documents (P4) and (P50)). Thus, the person skilled in the art had every reason to believe that a signal sequence from one eukaryote (human) would work in another eukaryot (yeast) ie. there existed a reasonable expectation that the proposed solution would succeed.

The skilled person would choose this solution rather than attempt to fit a yeast signal sequence in front of the human gene to be expressed because it required less manipulations.

- The experiment described in document (P3) concerning the failure to express rat growth hormone from a DNA sequence comprising the native rat signal sequence would not be considered relevant by the skilled person wishing to solve the above mentioned

problem. Indeed, of the three explanations which the authors provided for the observation, the inability to proceed normally through the secretion pathway was the only one which was speculative and not supported by any scientific facts. And besides, if the authors of document (P3) had had any doubts that secreting a human protein in yeast by using its native leader sequence could not be achieved, they would most probably not have made the statement mentioned above.

- The present case was alike to that dealt with in decision T 455/91 (see supra) where the claimed invention was a DNA vector suitable for use in expressing exogenous genes in yeast which differed from the vectors of the closest prior art in that they did not contain an ATG start signal, this signal being part of the exogenous DNA insert. In this earlier case, the then competent Board decided that the claimed vector lacked inventive step over the closest prior art in combination with the common general knowledge that such kinds of vectors existed for E.coli. The Board stated that what was required of the skilled person was only normal design procedures for which neither creative thinking nor inventive talent were necessary.

This conclusion equally applied to the present situation since the claimed vector only differed from those of the closest prior art in that they contained a signal sequence which was already known to fulfill its function across species barriers.

X. The Appellants requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request or first, second or third auxiliary requests, all submitted on 27 September 2002.

The Respondents requested that the appeal be dismissed

Reasons for the decision

Main request

Articles 123(2)(3) and 84 EPC

1. The deletion of the wording "heterologous to a yeast organism" in claim 1 avoids a redundancy since the protein to be expressed is already characterized as being human. The amended claim is, thus, clear. The amendment does not alter the findings in previous decision T 354/97 (see supra) that the requirements of Article 123(2)(3) EPC are fulfilled.

Article 56 EPC

2. The closest prior art is document (P3) which discloses the expression of **mature** human leukocyte interferon D in yeast cells from a recombinant vector wherein the DNA sequence coding for mature interferon is linked to a yeast promoter. As expected in the absence of a DNA encoding a leader signal sequence in the construct, the interferon is produced **intracellularly** in the cytosol of the yeast cells. A further experiment is conducted which involves expressing the DNA comprising the sequence coding for the rat growth hormone together with its own leader signal sequence. In that case, no translation is observed. On page 722, left-hand column, the authors express the opinion that: *"The yeast system should be particularly advantageous for the synthesis of glycoproteins from higher organisms because...the secretory pathway follows the same general pathway used by animal cells.".*

3. Starting from document (P3), the problem to be solved may be defined as setting up an improved process for the production of a human protein in yeast.

4. The solution given in claim 1 is a process whereby the human protein is secreted from the yeast cells, which involves expressing the corresponding coding sequence fused to a secretion signal sequence which is **not of yeast origin.**

5. From the above mentioned passage of document (P3), it is readily apparent that the authors considered that secretion from yeast cells was a direction of research worthy of investigation when wanting to produce proteins from higher organisms. Furthermore, as one of the proteins studied in document (P3) is a human protein, the skilled person would understand the statement as applying, in particular, to human proteins. The state of the art on file also shows that secretion was in general sought after: document (P4) (page 3369, left-hand column) for example points out that: *"Recombinant DNA technology attempts to produce higher cell proteins in bacteria. Such proteins are simpler to detect and purify if they are secreted from the cell."*

In the Board's judgment, it was, thus, obvious to think of attempting the secretion of human proteins by yeast when wanting to produce them.

6. The proposed solution, however, is not secretion of human proteins from yeast cells but secretion of human proteins from yeast cells **using a secretion signal sequence which is foreign to yeast**. The state of the art on file relating to the use of a secretion signal foreign to the host cells for secreting a gene product from a higher organism is represented by documents (P3), (P7), (P31), (P4) and (P50) can be summarized as follows:

- As already mentioned in point 2 above, document (P3) discloses that the DNA encoding the rat growth hormone preceded by its own signal sequence is transcribed but not translated. The authors attribute the phenomenon to the facts that the signal sequence may be inadequate to help the progression of the protein through the yeast secretory pathway, alternatively, to the sequence context preceding the initiator ATG or to codon usage differences (page 722, left-hand column).

- Document (P7) relates to the expression of ovalbumin in yeast. It is said on page 166 that *"investigations on the secretion of OLP in yeasts are hampered by the relatively low levels of OLP synthesis".*

- Document (P31) discloses that expression of the DNA encoding the human fibroblast interferon gene preceded by its own coding sequence in E.coli does not lead to the production of active fibroblast interferon. It is stated on page 5233, left-hand column: *"It is possible that preF-IF is exported to the periplasm with or without concomittant cleavage of its leader sequence and is rapidly destroyed there."*

- Document (P4) shows that the signal sequence of the rat preproinsulin allows secretion of the hormone in E.coli. This result is confirmed in document (P50) using a different construct.

7. In the Board's judgment, the teaching of document (P3) would deter the skilled person from attempting to secrete human proteins from yeast by using their own signal sequences. The Respondents argued that "the faulty secretion signal sequence" was the only one of the three reasons given for the failure in translation which was speculative and not supported by scientific

facts and thus, that it would not be taken seriously. This argument, however, is not found convincing: all three reasons are speculative before one of them is proven right. Moreover, the fact that the efficacy of the sequence context preceding the initiator codon and the effect of codon usage differences may be more easily tested than the adequacy of the secretion signal sequence does not mean that the signal sequence is less likely to be the reason why no translation is observed. Finally, the sentence in document (P3) mentioned in point 2 above implies that it should be feasible to secrete proteins of higher organisms from yeasts, yet it is not in any way indicative of which signal sequence may be useful.

8. Document (P7) reports difficulties in synthesizing a foreign protein in yeast. Document (P4) and (P31) disclose opposite results, a deficiency in the secretion pathway being mentioned in document (P31) as a possible explanation for the failure of producing an active protein.

9. Thus, it is concluded that the results reported in the prior art are not so clear-cut that they would give the skilled person wanting to secrete a human protein from yeast a reasonable expectation of success that it could be achieved with a signal sequence foreign to yeast.

10. The Respondents also emphasized that at the priority date, the secretion pathway was considered to be universal. It is indeed stated on page 466 of document (P53) that: *"Our results support a model of secretion in yeast that is strikingly similar to that observed in mammalian cells".* The results which are made mention of, concern the morphology of the secretory organelles, the division of the glycosylation steps between the ER and the Golgi apparatus and the location of energy-requiring steps (page 466, right-

hand column). Thus, they relate to the tertiary organisation and functionality of the cellular compartments and organelles involved in secretion and not to their structures at the molecular level. Accordingly, document (P53) cannot be read as suggesting that the signal sequence of the protein to be secreted which is expected to interact with some specific molecules belonging to the cellular compartments and/or organelles will necessarily be interchangeable between yeast and other higher organisms.

11. Other documents underscore the **relative functional** universality of the secretory pathways (document T-9) or **suggest** that the bacterial and eucaryotic presequences play similar and interchangeable roles on the basis of experiments carried out **only** in procaryots (document (P4)).

12. In contrast to these statements which are essentially of a predictive nature, it must be kept in mind that at the priority date, secretory signal sequences of yeast origin **were already characterized** as well as the corresponding DNAs (see, for example, document (P19)). Thus, in the Board's judgment, the skilled person knowing that the yeast secretory signal sequence would function in yeast, would choose the approach which consisted in fusing the DNA encoding the human protein to that encoding a yeast signal sequence when wanting to secrete the human protein from yeast. The Respondents' argument in this respect that the experimental steps to be taken to accomplish the fusion would have been considered too difficult cannot be followed, because at the priority date, it was already a matter of common knowledge to link together two DNA fragments in the required way (see for example, document (P20) where the rat preproinsulin gene is fused in a precise manner to all of, half of or only

the first four amino acids of a bacterial signal sequence).

13. In case T 455/91 (see supra), the problem to be solved was to construct vectors suitable for expressing any exogenous gene in yeast. The Board decided that it was obvious to bring an ATG codon into the expression vector as part of the 5' end of the exogenous gene taking into account the common general knowledge that ATG was universally needed for translation to be initiated and that vectors such as claimed had already been constructed for use with E.coli. They, thus denied inventive step. In the present case, the common general knowledge with regard to secretion is that a signal sequence is always required. The state of the art on file, however, fails to demonstrate that, as for translation initiation codon, the structure of signal sequence is identical in all organisms. The use in E.coli of vectors comprising a foreign signal sequence leads to inconclusive results (see point 6 above). Thus, the factual situations in the two cases being different, the reasoning on inventive step in case T 455/91 (see supra) cannot be applied in the present case.

14. For the reasons given in points 6 to 12 above, the skilled person would not have derived the presently claimed solution in an obvious manner from the prior art and, thus, inventive step is acknowledged.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The matter is remitted to the first instance with the order to maintain the patent on the following basis:

   Claims: claims of main request submitted on 27 September 2002.

   Description: pages 2 and 3 as submitted in the main request on 27 September 2002,
   pages 4 to 16 as granted.

   Figures: as granted.

The Registrar: The Chairwoman

P.Cremona U. Kinkeldey