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

Publication in the Official Journal ~~Yes~~ / No

File Number: T 158/91 - 3.3.2

Application No.: 84 302 725.1

Publication No.: 0 127 305

Title of invention: Human growth hormone, and process for its production, expression vehicles for producing pre HGH and host cells transformed thereby

Classification: C12N 15/00

D E C I S I O N
of 30 July 1991

Applicant: Genentech, Inc.

Headword: Human growth hormone/GENENTECH

EPC Article 54

Keyword: "Novelty - (no) - Technical teaching in a prior art document made available to the public"

Headnote

Case Number : T 158/91 - 3.3.2

D E C I S I O N
of the Technical Board of Appeal 3.3.2
of 30 July 1991

Appellant : Genentech, Inc.
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Decision under appeal : **Decision of Examining Division of the European Patent Office dated 28 June 1990 and posted on 31 July 1990 refusing European patent application No. 84 302 725.1 pursuant to Article 97(1) EPC.**

Composition of the Board :

Chairman : P.A.M. Lançon
Members : U.M. Kinkeldey
M.K.S. Aúz Castro

Summary of Facts and Submissions

I. European patent application No. 84 302 725.1, published under No. 0 127 305, was refused by the Examining Division. The refusal was based on 16 claims. Claim 1 reads as follows:

"1. A process for the preparation of mature human growth hormone (hGH), which comprises (i) expressing in a transformant prokaryotic host cell DNA encoding a pre-protein of hGH containing a signal sequence which, in its native environment, effects secretion of the desired protein and is endoproteolytically cleaved away within the host, and (ii) extracting from the cell culture mature hGH free from mature hGH having an extraneous N-terminal methionine."

II. The grounds given for refusal were that the application did not meet the requirements of Article 54(2) EPC. The arguments can be summarised as follows:

(a) The prior art document EP-A-0 038 182 (document (7)) aimed at providing a method for producing in a prokaryotic host a selected protein in mature form, that is a form which is free of fused sequences or other chemical substituents, such as f-met, so as to avoid any further treatment thereof.

As a solution to this technical problem said document proposed a method in which prokaryotic host cells were transformed with a vector in which the DNA encoding said selected protein included also an appropriate signal sequence (e.g. the protein's own signal sequence) that was cleaved of upon secretion through the host's membrane. The method, which was meant to be of general applicability, was

illustrated by examples in which it was shown that proinsulin could indeed be correctly processed and transported to the bacterial periplasm when a DNA sequence encoding the pre-protein (preproinsulin) was expressed in the host.

In Claim 6 the method was claimed with respect to specific proteins for which the corresponding DNA sequences were available at the time of filing, including prehuman growth hormone.

The quoted document affected directly the novelty of Claims 1 to 3 and, consequently, also of Claims 9 to 12, the latter being directed to the transformed host per se.

(b) The Examining Division was well aware of the fact that a document could be used for a novelty attack only if it contained an enabling teaching. It also agreed with the view of the applicant's representative that enablement involved not only availability of tools, but also sufficient guidance to put the invention into practice with a reasonable expectation of success and without undue experimentation. These criteria were satisfied in document (7) because the teaching given in this document had to be considered within the framework of the general knowledge that almost all secreted proteins, both eukaryotic and prokaryotic, had an amino terminal extension, or pre-sequence, or a signal, that was removed as or after the protein crossed the membrane. The aim was, in fact, to confirm this recombinant DNA system. In document (7) it was established that bacterial signal peptidases indeed recognised the eukaryotic clipping site and processed correctly the hybrid pre-protein

(preproinsulin) to proinsulin. This allowed the general conclusion that if a gene for a pre-protein was inserted downstream from a bacterial promoter in a recombinant DNA expression system, the mature protein, without extraneous bacterial amino acids, could be isolated from the bacterial periplasmic space.

(c) The generalisation in document (7) of the specific teaching directed to the expression of preproinsulin to any protein and the extension to other proteins for which the DNA coding sequences (tools) were available, including prehuman growth hormone seemed to be justified by the importance of their discovery and by the experimental evidence given (decision T 292/85, OJ EPO 1989, 275). Sufficient guidance was given in the specification to enable the skilled man to extend the special teaching to the whole of the field claimed, in particular to human growth hormone, by using routine methods of experimentation with a reasonable expectation of success.

(d) Admittedly, no special measures requiring inventive ingenuity were necessary in the present case in order to achieve the desired result since this depended simply on the combination of the human growth hormone with its own or other signal pre-sequences. This was exactly the teaching of document (7) and the present application confirmed indeed its validity.

(e) The fact that, in another prior art document, (Taniguchi et al., PNAS, USA Vol. 77, No. 9, September 1980, 5230, document (2)) it was disclosed that it was not possible to find processed fibroplast interferon in the periplasmatic space of E.coli cells, could not be interpreted as discrediting the

enabling disclosure of document (7). Rather the pre-fibroplast interferon, described in document (2) was indeed known to be quite unstable and thus it was not surprising for the skilled person that the hyperlabile protein could not be identified in the periplasm. Rather, later evidence had in fact confirmed that the conclusions drawn in document (7) were generally correct in spite of reports of cases in which no processing could be observed. As an expert opinion a publication in 1983 by Harris, Genetic Engineering, Vol. 4, Academic Press, London, pp. 131-133, 164-169 (document (6))was quoted.

(f) Also several further documents which in the applicants' representative's opinion should be considered for a decision on a possibly enabling effect of the teaching of document (7) could not cause any doubt about the general applicability of the approach of document (7) or indicate the existence of a prejudice away from accepting it. The references were either totally irrelevant since they were concerned with different subject-matters or did not at all dispute the validity of the teaching of document (7), and in particular did not exclude the possibility of correct processing and secretion. In fact, for example document (11) (Nucl. Ac. Res., Vol. 9, No. 1, 1981, 19) mentioned rapid turnover or degradation as possible causes for the failure to find the mature protein in the periplasmatic space and thus did not at all exclude the possibility of correct processing and secretion.

(g) It was therefore concluded that the teaching in document (7), in consideration of the state of the art at the date of filing of the present application, was such as to enable the skilled person to carry out

the technical teaching disclosed therein with respect to the non-specifically exemplified embodiment of human growth hormone. Thus, document (7) belonging to the state of the art, anticipates the matter of present Claims 1 to 3.

III. The Appellants appealed against this decision and paid the corresponding fee. They further filed the written statement setting out the grounds for appeal.

IV. Together with their statement of grounds they filed additional documents (14) to (21) in support of their arguments and as evidence that both before and after the publication date of document (7) those skilled in the art were using alternative and more cumbersome strategies to obtain mature human growth hormone.

V. Oral proceedings took place on 30 July 1991.

VI. The arguments put forward by the Appellants during the appeal proceedings can be summarised as follows:

(a) A proper analysis in the present case had to consider the art published between the publication date of document (7) and the filing date of the present application. In this context it was important to emphasise the real-world situation regarding the secretion and export of eukaryotic proteins in E.coli. Many proteins had been reported as being found in the cytoplasm of E.coli. A few had been poorly exported, as shown at the end of Table 2 in document (6). From more recent disclosures it had to be concluded that it was still unpredictable today whether a different protein will be exported from a cell without empirical testing. In order for proteins to be exported they must remain in an unfolded state

following synthesis on the ribosomes. Folding destroyed their exportability. To keep in an unfolded state, molecules, functionally termed chaparones, had to attach to the new protein and prevent folding. The signal sequence then contacted the transport system, and as the protein was exported the chaparones were removed. Consequently, as the protein moved through the membrane it began to fold because the required chaparones were not present in the E.coli periplasmic space. Some proteins, for unknown reasons, failed to bind to chaparones and thus folded before they could be exported. They were then found in the intracellular region rather than exported. Apparently, a very slight change in the amino acid sequence could profoundly change the ability of the limited number of chaparones to bind to the eukaryotic protein and to inhibit folding. Hence, the export of recombinant eukaryotic proteins in E.coli remained arbitrary and unpredictable up to this day.

(b) Therefore, those skilled in the art would not have taken the speculation in document (7) as reliable so far as secretion and processing were concerned. They would have recognised the listing of specific proteins in document (7) as being arbitrary, based on commercial usefulness and the availability of their DNA, rather than a scientific invitation to try those in particular. In view of the results produced in document (2) on one of the proteins speculated about in document (7), namely the preinterferon, and the negative results obtained with rat, porcine and bovine pregrowth hormones as disclosed in documents (10) and (14), they would have had no reasonable expectation of success with human growth hormone.

(c) Thus, document (7) did not provide a disclosure which would have enabled a skilled person to produce by recombinant DNA technique mature human growth hormone free from mature human growth hormone having an extraneous N-terminal methionine, as claimed in the present application, and consequentely was not made available to the public as required by Article 54(2) EPC.

VII. The Appellants requested that the decision under appeal be set aside and that a patent be granted on the basis of Claims 1 to 16 filed with a letter dated 17 January 1989.

Reasons for the Decision

1. The appeal is admissible.

2. Novelty (Article 54(1) and (2) EPC)

2.1 The question at issue is whether or not the claims on file are novel in the light of document (7). Document (7) relates to the expression of a protein containing a leader sequence and transported into the periplasmatic space, being rat proinsulin and preproinsulin. On page 1 of the description of the published application in the paragraph "field of the invention" it is said that the invention of document (7) generally relates to a method of synthesising within a bacterial host, and secreting through the membrane of the host, a selected protein or polypeptide. The protein or polypeptide is then further specified as being for example a eukaryotic cell protein, e.g. human growth hormone. This teaching is further reflected in Claim 6 and Claim 14, the latter relating to a host, characterised in that the non-bacterial DNA fragment codes inter alia for prehuman growth hormone.

When comparing this teaching to the subject-matter of Claim 1 (see paragraph I above) it has to be realised that they are identical.

2.2 The Board agrees with the Examining Division's and the Appellant's position that the teaching of document (7) had to be available to the public at the priority date of the present application as to represent prior art within the meaning of Article 54(2) EPC. According to established case law of the Boards of Appeal (cf. T 206/83 OJ EPO 1987, 5), which is followed by this Board and to which both the Examining Division and the Appellants agree, the criteria for examining the reproducibility of a certain technical teaching are the same in cases where the disclosure of a prior art or a disclosure of a patent application in question has to be judged.

2.3 Certainly, the question of sufficient disclosure, be it of a prior art document or a patent application in question, has to be examined in each case on its own merits. An examination as to the sufficiency of a disclosure depends on the correlation of the facts of the case to certain general parameters.

These parameters are for example:

(a) the character of the technical field and the average amount of effort necessary to put into practice a certain written disclosure in that technical field;
(b) the time when the disclosure was presented to the public and the corresponding common general knowledge;
(c) the amount of reliable technical details disclosed in a document.

2.4 As to parameter (a) above the Board notes that the subject-matter of document (7) belongs to a technical field which is acknowledged as a difficult and complex technical field. The average amount of time and effort to reproduce certain recombinant DNA processes for the expression of protein is usually very high. The Board is well aware of the fact that on the basis of a publication describing a successful expression no reliable predictions are possible as to whether the repetition of this teaching would also lead to successful expression of another gene prepared by recombinant DNA technique.

In the present case, however, the DNA sequence of human growth hormone was already known, so that inter alia from the knowledge of certain restriction sites a reasonable extrapolation with respect to a successful application of the recombinant DNA technique described in document (7) was possible.

2.5 As to parameter (b) above the priority date of the present patent application is 23 April 1983. At that time the techniques in the field of molecular genetics developed from its infancy and became routine work for the basic tools and process steps of genetic engineering, though still remaining difficult, complex, time-consuming and not entirely predictable. The Board agrees to the Appellants' position that an answer to the question whether the disclosure of document (7) enabled a skilled person to put into practice the written teaching, common general knowledge has to be considered which is said to be reflected by numerous documents filed by the Appellants during the examining procedure and, in particular during the appeal proceedings. As far as the documents filed during the examining proceedings are concerned the Board agrees to the judgment given by the Examining Division (see above paragraph II (e) and (f)).

As far as the documents filed during the appeal proceedings are concerned, the Board is of the opinion that their disclosure does not give rise to doubts about the information about common general knowledge represented by the documents discussed by the Examining Division. Thus, the Board confirms the Examining Division's view that the fact that the mentioned documents provide information that the teaching of document (7) did not work in each and every case does not mean that the disclosure of document (7) did not work in a sufficiently reliable manner in other cases. The Appellants do not deny that at least in some cases the teaching of document (7) works.

2.6 As to the technical details disclosed in (7) (see parameter (c) above), the Board notes that the description of document (7) provides a detailed route for the isolation and construction of a suitable plasmid for the expression of the desired protein whereby the plasmid is isolated from a certain strain of the bacterium E.coli K-12 under conditions described precisely; the isolated plasmid was tailored by cutting or cleaving the plasmids with certain restriction enzymes and plasmid fragments ligated as desired and finally a DNA sequencing was carried out. All these steps are described in a detailed manner, for example, in steps A to E on pages 17 to 19 of the published document (7).

In a paragraph headed by the sentence "Making the non-bacterial DNA fragments to be cloned into the cloning vehicles (VI)" a technically detailed description is provided how to obtain the DNA sequence containing the gene for rat preproinsulin. This part of the description of document (7) certainly is directed to the preparation of a DNA sequence which is characterised by coding for the

protein rat preproinsulin. Since genes coding for different proteins also differ from each other, this part of the description in document (7) has to be modified and adopted in the case for a gene coding for prehuman growth hormone. It is evident that in the case of different DNA sequences with respect to a gene coding for human pregrowth hormone instead of rat preproinsulin, different endonuclease restriction sites may occur than those mentioned in this particular part of the description in document (7). Although the parameters for a successful construction of the respective DNA sequence are complex and may not in each and every case of a protein lead to success, the Board comes to the conclusion that the mentioning of the protein human growth hormone in document (7) apparently was not done merely speculatively, as emphasised by the Appellants, but rather that the selection of proteins in document (7) as being suitable for an analogous process as described in detail for the protein rat preproinsulin was based on such proteins whose DNA sequence was already known or which were otherwise investigated to such an extent that an extrapolation was reasonable from the details given for the preparation of the gene coding for rat preproinsulin to the genes coding for the other proteins mentioned in document (7).

2.7 Thus, although the description of document (7) does not provide precise technical steps for the preparation of the gene coding for human pregrowth hormone, the Board believes that a skilled person was equipped with the knowledge of the already known DNA sequence for human growth hormone and his common general knowledge in connection with the detailed description in document (7) as far as the other tools were concerned. The Appellants did not claim that any particular tools, extending common general knowledge or the technically detailed description of document (7) were necessary to carry out the process

as it is subject-matter of the present application. He was reliably able to put into practice the synthesis within a bacterial host and secreting through the membrane of the host a selected protein or polypeptide whereby this protein was human pregrowth hormone. One may, therefore, consider the present application as a confirmation that the process as described in detail in document (7) for the case of rat preproinsulin and more generally for human pregrowth hormone does function.

2.8 The Board, therefore, comes to the same result as the Examining Division as to the judgement that the technical teaching of document (7) was made available to the public.

2.9 As already stated above in paragraph 2.1, document (7) describes identically the same subject-matter as is claimed in Claim 1 of the present application. Consequently, the subject-matter of the latter is not novel.

Thus, the Board confirms the decision of the Examining Division in its entirety.

Order

For these reasons, it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

P. Martorana P. Lançon