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Veröffentlichung im Amtsblatt	Ja/Nein
Publication in the Official Journal	Yes/No
Publication au Journal Officiel	Oui/Non

Aktenzeichen / Case Number / N° du recours : T 347/87 - 3.3.2

Anmeldenummer / Filing No / N° de la demande : 80 103 748.2

Veröffentlichungs-Nr. / Publication No / N° de la publication : 0 022 242

Bezeichnung der Erfindung:
Title of invention:
Titre de l'invention : Cloning vehicle, method of constructing it, method of producing human growth hormone and bacteria therefor.

Klassifikation / Classification / Classement : C12N 15/00

ENTSCHEIDUNG / DECISION
vom / of / du 18 July 1990

Anmelder / Applicant / Demandeur : Genentech, Inc.

Patentinhaber / Proprietor of the patent /
Titulaire du brevet :

Einsprechender / Opponent / Opposant :

Stichwort / Headword / Référence : Cloning vehicle/GENENTECH

EPÜ / EPC / CBE Articles 54, 56, 83, 84, 111(1)

Schlagwort / Keyword / Mot clé :
"Sufficiency and clarity (yes)"
"Novelty (yes)"
"Inventive step - method claims concern non-obvious alternative"
"Remittal of the case for further prosecution"

Leitsatz / Headnote / Sommaire

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Case Number : T 347/87 - 3.3.2

D E C I S I O N
of the Technical Board of Appeal 3.3.2
of 18 July 1990

Appellant : Genentech, Inc.
460 Point San Bruno Boulevard, So.
San Francisco, California 94080
(US)

Representative : Patentanwälte Dipl.-Ing. A. Grünecker
Dr.-Ing. H. Kinkeldey
Dr.-Ing. W. Stockmair
Dr. rer. nat. K. Schumann
Dipl.-Ing. P.H. Jakob
Dr. rer. nat. G. Bezold
Maximilianstrasse 58
D-8000 München 22

Decision under appeal : Decision of Examining Division 023 of the European Patent Office dated 25 February 1987 refusing European patent application No. 80 103 748.2 pursuant to Article 97(1) EPC

Composition of the Board :

Chairman : P. Lançon
Members : A. Nuss
R. Schulte

Summary of Facts and Submissions

I. European patent application No. 80 103 748.2 (publication No. 0 022 242) was refused by the decision of the Examining Division of the European Patent Office dated 25 February 1987. The decision was based on Claims 1 to 27, whereby

- Claims 1 to 9 concerned a method of constructing a replicable cloning vehicle comprising a quasi-synthetic gene coding for a particular polypeptide of known amino acid sequence, the gene being characterised as product-by-process;

- Claims 10, 11, 13 to 20 and 24 concerned a series of plasmids (some of which were characterised by code names such as pGH6, pGH107 and pGH107-1), whereby Claim 13 was worded as follows:

  "A replicable bacterial plasmid capable, in a transformant bacterium, of expressing human growth hormone unaccompanied by extraneous conjugated protein".

- Claims 12, 21, 22, 23 and 25 concerned a method of producing a polypeptide employing a cloning vehicle, a viable bacterial culture of bacterial transformants comprising certain plasmids and a method of producing human growth hormone based on such a culture;

- Claims 26 and 27 concerned a replicable cloning vehicle capable of expressing a mammalian polypeptide.

II. The refusal was based on the following three grounds:

(i) non-compliance of Claims 13 to 16, 20, 21, 26 and 27 with Rule 29(1), first sentence, in connection with Article 84 EPC, first sentence and Rule 27(1)(d), first half sentence. These claims were considered to merely paraphrase, in a broad and general language, technical problems which appeared to be already known from document (I) EP-A-0 001 929 without indicating technical features ascribable to what might be considered a solution thereof;

(ii) non-allowability of Claims 1 to 6 under Article 56 EPC essentially for the reason that it was known from document (I) that the heterologous DNA, coding for a polypeptide like HGH, could comprise in addition to synthetic DNA (DNA made in vitro) also cDNA resulting from reverse transcription from mDNA;

(iii) non-allowability of Claims 1 to 9, 10, 22, 23 and 25 under Article 83 EPC, since in the present application the starting material appeared to be a tumor of an individual human leading to plasmid pHGH 31, a specific but not completely specified DNA sequence due to the known allelic phenomenon.

In view of the objection made under (i), it was left undecided in the decision whether the subject-matter of the claims concerned was novel or not. For Claims 1 to 12, 17 to 19 and 22 to 25, novelty was however acknowledged.

III. The Appellant lodged an appeal against this decision.

IV. An oral hearing was held on 18 July 1990. During the course of the hearing a new request with revised claims (one set of claims for the Contracting States NL and SE, the other set for the Contracting State AT) was submitted on behalf of the Appellant to replace all earlier requests. Each set of claims comprised 24 claims.

- The claims for NL and SE are as follows (the bold parts mark noticeable differences in wording vis-à-vis the claims considered in the contested decision):

"1. A method of constructing a replicable cloning vehicle capable, in a microbial organism, of expressing a particular polypeptide of known amino acid sequence wherein a gene coding for the polypeptide is inserted into a cloning vehicle and placed under the control of an expression promoter,
characterized in that
the gene coding for the polypeptide is prepared by
(a) obtaining by reverse transcription from messenger RNA a first gene fragment for an expression product other than said polypeptide, which fragment comprises at least a substantial portion of the coding sequence for said polypeptide;
(b) where the first fragment comprises protein-encoding codons for amino acid sequences other than those contained in said polypeptide, eliminating the same while retaining at least a substantial portion of said coding sequence, the resulting fragment nevertheless coding for an expression product other than said polypeptide;
the product of step (a) or, where required, step (b) being a fragment encoding less than all of the amino acid sequence of said polypeptide;

(c) providing by organic synthesis one or more gene fragments encoding the remainder of the amino acid sequence of said polypeptide, at least one of said fragments coding for the amino-terminal portion of the polypeptide; and

(d) deploying the synthetic gene fragment(s) of step (c) and that produced in step (a) or (b), as the case may be, in a replicable cloning vehicle in proper reading phase relative to one another.

10. An expression plasmid comprising functional genes for ampicillin and tetracycline resistance and, lying between said genes, a lac promoter system oriented to promote expression of a polypeptide of known amino acid sequence in the direction of the gene for tetracycline resistance and restriction sites being positioned downstream from said promoter
characterized in that
said promoter is a tandem lac promoter and the restriction sites comprise sites, yielding blunt ends upon cleavage, and permitting proper inserting of heterologous DNA, coding for said known amino acid sequence, between said sites so as to come under the control of said promoter system.

11. The plasmid pGH6 (ATTC, No. 40012)
characterized in that
it is obtainable from known pBR322 by inserting a 285 base pair Eco RI fragment known from plasmid pKB268, containing two 95 base pair UV5 lac promoter fragments separated by a 95 base heterologous DNA fragment, into the Eco RI site of pBR322 in proper reading phase with the gene for tetracycline resistance, and destroying the

tetracycline gene distal Eco RI site by partial Eco RI digestion, repair of the resulting single-stranded Eco RI ends with DNA polymerase I and recircularization of the plasmid by blunt-end ligation.

12. A replicable bacterial plasmid obtainable according to a method of one of Claims 1 to 9 capable, in a transformant bacterium, of expressing human growth hormone unaccompanied by extraneous conjugated protein, and in which the human growth hormone encoding gene is under the control of tandem lac promoters.

16. The plasmid pHGH107 (ATCC, No. 40011), characterized in that it is obtainable from plasmid pGH6 (ATCC, No. 40012) by treating pGH6 successively with Hind III, nuclease S1 and Eco RI, and ligating the resulting vector, having one Eco RI cohesive end and one blunt end, with a 591 base pair Eco RI/SmaI fragment of human growth hormone DNA.

17. The plasmid pHGH107-1, characterized in that it is obtainable from plasmid pHGH107 (ATCC, No. 40011), by cleaving pHGH107 with Eco RI, digesting the resulting single-strand ends with S1 endonuclease and recirculating by blunt-end ligation with T_4 ligase.

24. A method of producing human growth hormone employing the cloning vehicle constructed according to Claim 7 including expressing the amino acid sequence of the human growth hormone free of extraneous N-terminal protein."

The further (in)dependent Claims 2 to 9, 13 to 15 and 18 to 23 correspond in substance to previous Claims 2 to 9, 14 to 16, 20 to 24 and 12. Previous Claims 25 to 27 had been deleted.

- The Claims for AT differ from those for the other Contracting States in that all of them but Claim 19, which concerns a viable bacterial culture, are drafted as method claims.

V. The Appellant submitted in the proceedings and at the oral hearing the following arguments:

(1) It was agreed that document (I) disclosed the "direct" expression of a polypeptide, i.e. "as such", and not as a fusion, despite the fact that this disclosure focused on expression of fusions. However, this document merely concerned a generic disclosure of how to express DNA sequences made by chemical DNA synthesis (synthetic gene) so as to produce polypeptides. In practice, the use of purely in vitro preparation of genes encoding desired proteins became actually an obstacle when envisaging the production of proteins that were substantially larger than somatostatin, e.g. growth hormones. At that time, synthesizing a gene adequate to encode a growth hormone nearly 600 bases in length, would have taken longer than one year and entailed considerable expense.

Moreover, long DNAs obtained as cDNA were frequently truncated at the 5′ end, whereby the 5′ terminus of the cDNA was often located at a point within the coding sequence, whereas intact cDNAs coding for a protein like growth hormone encoded preproteins containing extraneous N-terminal polypeptide.

(2) The passage in document (I) concerning the Ullrich et al. publication was misleading, because the problem there was to find a convenient technique for inserting cDNA into a vector. This problem was overcome by synthesizing a short DNA fragment containing a Hind III restriction site and ligating this "linker" to the ends of the cDNA. Thereafter both the vector and the ligated linker-cDNA construct were digested with cohesive termini generating Hind III and the two fragments thus obtained ligated to produce the final vector construct. Therefore, in this method the linker merely provided proper ligation sites to the cDNA and did not encode a substantial portion of the N-terminal sequence of a desired polypeptide. However, in the present application it was essential that the in vitro synthesized DNA encoded at least a part of the polypeptide desired to be produced, including the amino terminal portion of the polypeptide.

(3) As to the question of sufficiency of disclosure under Article 83 EPC, the application disclosed all relevant details for both the construction of a quasi-synthetic gene coding for a polypeptide like human growth hormone and the subsequent expression of the encoded protein. Moreover, the newly constructed plasmids had been deposited with ATCC and the starting material, i.e. pituitary tissues, continued to be freely available to the man skilled in the art in any number. As to allelic variations of the latter, the man skilled in the art would be able to repair the DNA in order to conform exactly to the sequence set forth in the present application.

For the rest, it was already decided in decision T 292/85 that generally applicable biological processes were not insufficiently described for the sole reason that some starting materials or genetic precursors therefor, e.g. a DNA or a plasmid, were not readily available to obtain each and every variant of the expected result of the invention, provided the process as such was reproducible.

VI. The Appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of the two sets of Claims 1 to 24 (one set for NL and SE, the other set for AT), filed during oral proceedings.

Reasons for the Decision

1. The appeal is admissible.

2. The amendments which are incorporated in the present claims (see bold parts of the claims under IV above) are not such that the application contains subject-matter which extends beyond the content of the application as filed (Article 123(2) EPC). In particular, Claims 1 to 24 for NL and SE are supported by the original disclosure as follows:

- Claims 1 to 9 correspond in substance to Claims 1 to 9 as originally filed,

- Claim 10 differs from original Claim 20 essentially in that it is now specified, instead of being implied, that the heterologous DNA codes for the desired polypeptide of known amino acid sequence to be

expressed (see also page 6, lines 24 to 32 and page 12, line 21 to page 13, line 23 of the original description),

- Claims 11, 16 and 17 correspond to original plasmid Claims 21, 15 and 16 completed by including additional characteristics of these plasmids from the original description (see page 18, lines 1 to 17; page 19, lines 10 to 29 and Figure 5; page 20, lines 3 to 15),

- Claim 12 differs from original Claim 10 in that it is now limited to a replicable bacterial plasmid obtainable according to a method of one of Claims 1 to 9, i.e. one including a quasi-synthetic gene, with the additional condition that the human growth hormone encoding gene is under the control of tandem lac promoters in accordance with the original disclosure (see in particular page 12, line 21 to page 13, line 23; page 20, lines 19 to 22 and page 18, lines 1 to 17 and original Claim 14),

- Claims 13 to 15, 18 and 22 cover further embodiments falling under present Claim 12, supported by original Claims 11 to 13, 22 (in combination with original Figures 1 and 3) and 19,

- Claims 19 to 21 correspond to original Claims 17 to 19,

- Claim 23 corresponds in substance to original Claim 23,

- Claim 24 is a new claim implied by and therefore derived from the whole original disclosure as such (see page 6, lines 23 ff.; page 8, lines 12 ff. and page 13, line 24 to page 20, line 15).

Similar considerations apply to Claims 1 to 24 for AT (see above, point IV, last paragraph).

3.1 The Examining Division refused previous Claims 13 to 16, 20 and 21 on the basis of objections made under Article 84 in combination with Rules 29(1) and 27(1)(d) EPC (see point II(i) above). These claims correspond to a great extent to present Claims 12 to 15, 18 and 19, whereby Claim 12 is actually a substantially limited version of old Claim 13 in which some of the essential technical features of the plasmid are described by way of functional terminology such as "... capable ... of expressing human growth hormone unaccompanied by extraneous conjugated protein". Obviously, a limitation of this plasmid, defined in functional terms, to the specific embodiments described in the application (i.e. plasmids pHGH107 and pHGH107-1) would render illusory effective patent protection. However, as already decided earlier by this Board in a quite similar case, functional terminology as such is not objectionable when it is otherwise not possible to assure fair protection having regard to the nature of the alleged invention (see decision T 292/85, "Polypeptide expression/GENENTECH I", OJ EPO 1989, 275, in particular point 3.1.2 of the Reasons). Therefore, also those claims involving functional terminology must be regarded under the EPC as defining the matter for which protection is sought in terms of the technical features of the claimed invention.

The further embodiment according to present (dependent) Claim 13 (i.e. previous Claim 14) that "the human growth hormone encoding DNA comprises in substantial proportion cDNA or a replication thereof", merely expresses that the encoding DNA (gene) is not entirely composed of cDNA. If this were the case, the whole gene would be derived from

DNA obtained by reverse transcription from messenger RNA and it would thus not be possible to complete its encoding part by synthesis, as is required in the present application (see in particular Claim 1 and page 6, lines 24 ff.). Consequently, Claim 13 concerns a feature of technical nature which causes no difficulty of interpretation, either taken alone or in combination with Claim 12 on which it depends.

For the reasons already indicated above, the Board sees no illicit formulation in the functional terminology used in dependent Claims 14 and 15 (previous Claims 15 and 16), specifying that in a method according to Claims 12 or 13, the plasmid exhibits resistance to at least one antibiotic and, possibly, lacks the tet promoter, but yet exhibits tetracycline resistance.

3.2 A further objection of the Examining Division was that the broad and general language of these claims would lead to embrace solutions which were not provided in the disclosure of the present application and which, therefore, remain to be solved in the future. This objection actually suggests that claims drafted in essentially functional terms are not properly supported in their scope. However, as set out in detail in the previous decision T 292/85 (see above), there is no legal basis for such objections (see point 3.1 of the Reasons).

4. In the decision under appeal, the objection under Article 83 EPC was based on the nature of the starting material used in the application, viz. a pituitary growth hormone-producing tumor of an individual human, leading to plasmid pHGH31. This plasmid was considered to be a specific, but not completely specified DNA sequence, due to the known allelic phenomenon. The Examining Division required thus identical repeatability of this plasmid, a

prerequisite for obtaining derived plasmids pHGH107 and pHGH107-1. The present case is also in this respect not different from the earlier case T 292/85 (see above), where the Board held that the disclosure was sufficient in respect of the preparation of human hormones, although each person, as a source, could only provide an individual variant of the DNA precursor of the hormone, without of course any guarantee that each and every variant of the expected result of the invention, e.g. the product, could be obtained or that such source would remain available to the public, provided the process as such always worked (see in particular points 3.3.2 and 3.3.3 of the Reasons).

Under these circumstances, the objection under Article 83 EPC are not considered as founded.

5. It was left undecided by the Examining Division whether the subject-matter of previous Claims 13 to 16, 20 and 21 met the requirements for novelty or not. As already pointed out earlier (see point IV of the Summary of Facts and Submissions, and points 2 and 3 of the Reasons), present Claims 12 to 15, 18 and 19 differ from these previous claims in that Claim 12 is now limited to a replicable bacterial plasmid obtainable according to a method of one of Claims 1 to 9, i.e. one including a quasi-synthetic gene, with the additional condition that the human growth hormone encoding gene is under the control of tandem lac promoters. However, in document (I) no reference to a tandem lac promoter can be found, so that at least this additional feature clearly distinguishes the claimed plasmid from those described in the prior art document. The same distinction can be found in Claim 10 (differs from previous Claim 10 only by the presentation in two-part form) for which no novelty objection was raised by the Examining Division. Hence, the

subject-matter of Claim 12 as well as that of dependent Claims 13 to 15, 18 and 19, is novel.

As to the remaining claims, novelty was actually acknowledged by the Examining Division. The Board has thus no reason to call into question this assessment.

6. Another ground for the refusal of the application concerned the claimed method of constructing a replicable cloning vehicle in view of the expression of a particular polypeptide of known amino acid sequence, whereby the Examining Division relied on document (I) to deny any inventive merit of Claims 1 to 6.

6.1 Document (I) is clearly the closest state of the art. This document describes a recombinant bacterial plasmid suited for transformation of a bacterial host and use therein as a cloning vehicle, wherein the plasmid comprises:

(i) a regulon homologous to the bacterial host in its untransformed state; and

(ii) in reading phase with the regulon, a DNA insert (gene) coding for any heterologous polypeptide of known amino acid sequence, such that bacteria transformed by the plasmid are capable of expressing said amino acid sequence in recoverable form and whereby the expressed heterologous polypeptide may be a mammalian polypeptide such as somatostatin or human growth hormone.

There, the genes coding for heterologous polypeptide may be prepared by chemical DNA synthesis, wherein codons according to the genetic code are chosen and assembled in the desired sequence. Where the structural gene of a desired polypeptide is to be inserted in a cloning vehicle

for expression as such, the gene is preceded by a short codon (e.g. ATG) and immediately followed by one or more termination or stop codons. However, in addition to DNA made in vitro, the heterologous DNA may comprise cDNA resulting from reverse transcription from mRNA. The aim behind all this is to make possible expression of a functional polypeptide product from a synthetic gene (see in particular page 6, lines 2 to 4 and lines 20 to 40; page 8, line 25 to page 9, line 6; page 10, lines 21 to 26 and page 11, lines 14 to 30).

According to the Appellant, the use of these known purely in vitro methods for preparing genes encoding proteins substantially larger than somatostatin, such as growth hormones composed of nearly 600 bases in length, became an obstacle when trying to synthesize large genes (see point V(1) above).

6.2 The problem vis-à-vis the closest prior art document (I) is to be seen in providing an alternative route for the preparation of a replicable cloning vehicle capable, in a microbial host, of expressing a gene coding for a particular polypeptide of known amino acid sequence.

In order to solve this problem, Claim 1 as now on file proposes a method wherein the gene inserted into the cloning vehicle is a quasi-synthetic gene, prepared in accordance with steps (a) to (d) indicated in the claim, which is composed of a mRNA transcript fragment coding for at least a substantial portion of the polypeptide to be expressed and one or more gene fragments encoding the remainder of the amino acid sequence of said polypeptide, at least one of said fragments coding for the amino-terminal portion of the polypeptide.

The experimental part of the application (see page 13, line 24 to page 22, line 19 and Figures 1 to 5) shows that the said problem was indeed solved by this proposal.

6.3 The Examining Division objected in its decision that it was already mentioned in document (I) that the heterologous DNA, coding for a polypeptide like human growth hormone, could comprise not only synthetic DNA (i.e. DNA made in vitro), but also cDNA resulting from reverse transcription from mRNA. The assessment was based on the statement in the description that "in addition to DNA made in vitro, the heterologous DNA may comprise cDNA resulting from reverse transcription from mRNA". However, it is not mentioned in the decision that this statement was immediately succeeded by brackets containing a reference to the following publication: Ullrich et al, Science 196, 1313 (1977). The exact meaning of the statement can thus be established on the basis of the original publication on which it relies.

As correctly pointed out by the Appellant, Ullrich et al were confronted with a problem different from that of the present application, viz. that of finding a technique for inserting rat insulin cDNA into a vector, whereby the solution consisted in using chemically synthesized linkers containing a Hind III restriction site and ligating these short DNA fragments to the ends of the cDNA. The next step consisted in the cleavage of the ligated linker-cDNA construct with cohesive termini generating endonuclease (Hind III), followed by ligation to similarly cleaved plasmid DNA (see page 1314, right column, last paragraph and Figure 1). Plasmid pAU-1, obtained by transformation with the total rat islet cDNA contained an inserted DNA fragment approximately 410 nucleotides in length, which was released from the plasmid by Hind III endonuclease digestion. This cloned DNA fragment, which hybridized to rat islet cDNA, was isolated and subjected to DNA sequence analysis, whereby a strand was determined with an

amino acid sequence which exactly corresponds to the entire coding region for rat proinsulin I and 13 out of 23 amino acids of the prepeptide sequence (see page 1316, left column, last paragraph to right column, penultimate paragraph and Figures 3 to 7).

The Board therefore concurs with the Appellant that in this publication the chemically synthesized DNA fragments are not part of the gene to be expressed. They serve as linkers providing proper ligation sites to the cDNA without exercising any coding function for the desired polypeptide. In the known construct, the inserted DNA fragment corresponds to the total rat islet cDNA comprising the entire coding region for rat proinsulin I and 13 out of 23 amino acids of the prepeptide sequence, whereas in the present case all leader or signal DNA is cleaved after which synthesis will restore only those codons required for expression of the complete (mature) polypeptide. Therefore, the reference to Ullrich et al in document (I) could by no means suggest the preparation of the quasi-synthetic coding gene of the present application.

It follows from the above, that the method of Claim 1 is not rendered obvious by the state of the art. The same applies to Claims 2 to 9 which depend on it.

Consequently, the subject-matter of Claims 1 to 9 of both sets of claims must be considered to involve an inventive step.

7. As to the remaining Claims 10 to 24, the question of inventive step stayed so far entirely open. Although the Board has, therefore, no intention of investigating this matter of its own motion, it must be observed that the problem to be solved by at least some of these claims can

hardly be the one mentioned in point 6.2 above. The Board wondered whether sufficient attention was given to this before.

The case is remitted to the first instance in order to complete substantive examination (Article 111(1) EPC).

Order

For these reasons, it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the Examining Division for further examination on the basis of Claims 1 to 24 (one set for NL and SE, the other set for AT), filed during oral proceedings.

The Registrar: The Chairman:

M. Beer P.A.M. Lançon