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D E C I S I O N
of 14 December 2001

Case Number: T 0343/98 - 3.3.4

Application Number: 86904536.9

Publication Number: 0228449

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Language of the proceedings: EN

Title of invention:
Fibroblast growth factor

Patentee:
THE SALK INSTITUTE FOR BIOLOGICAL STUDIES

Opponent:
Scios Nova, Inc

Headword:
Fibroblast growth factor/THE SALK INSTITUTE FOR BIOLOGICAL
STUDIES

Relevant legal provisions:
EPC Art. 56, 108

Keyword:
"Appeal admissible - (yes)"
"Main request: inventive step (yes)"

Decisions cited:
-

Catchword:
-



Case Number: T 0343/98 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 14 December 2001

Appellant:
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Decision under appeal: Decision of the Opposition Division of the
European Patent Office posted 28 January 1998
revoking European patent No. 0 228 449 pursuant
to Article 102(1) EPC.

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: R. E. Gramaglia
 C. Holtz

Summary of Facts and Submissions

- I. With the decision under appeal of 28 January 1998, the Opposition Division revoked European patent No. 0 228 449 (application No. 86904536.9), which had been opposed by the respondent on the grounds of lack of novelty and inventive step, as well as insufficiency of disclosure.
- II. The appellant/patentee on 27 March 1998 filed a notice of appeal, requesting that the decision under appeal be set aside, that the opposition be dismissed and that the patent be upheld. The appeal fee was paid on the same date. On 7 April 1998, the appellant filed an amended notice of appeal, additionally requesting that the patent be upheld unamended or as amended in accordance with an auxiliary request, that oral proceedings be appointed, if the board could not allow the appeal on the written submissions, and finally that the appeal be subject to priority prosecution.
- III. On 5 June 1998, 16 pages, headed "URGENT FACSIMILE TRANSMISSION", were received by the EPO per fax. This submission stated that grounds of appeal were filed together with an affidavit. The number of pages of the fax was given as 85. Pages numbered 1-6 and 8-17 were received.
- IV. An investigation within the EPO as well as within the office of the appellant's representative was without result and the missing pages were not found. The appellant filed a complete set of grounds of appeal on 22 June 1998. As far as the first 16 pages are concerned, this submission contained changes on page 5 in comparison to the originally filed grounds, insofar as two points had been added, 0.1.2.1 and 0.1.2.2.

These points contained a reference to sworn testimony to which the appellant would refer and the indication of a further sworn testimony to be submitted shortly.

V. The board in a communication of 16 October 1998 expressed its opinion that the appeal was in existence since the notice of appeal and appeal fee had been submitted in due time. Further, the board was of the opinion that the grounds, as received in due time by the EPO, were admissible. Together with the detailed amended notice of appeal, the board was satisfied that the appeal as a whole was admissible.

VI. The patent was granted with 33 claims for all designated Contracting States except AT and 10 claims for AT. Claims 1 and 22 as granted for the non-AT designated Contracting States read as follows:

"1. DNA encoding the amino acid sequence of a mammalian basic fibroblast growth factor (bFGF) polypeptide containing the 146-amino acid residue sequence:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Pro-Ala-Leu-Pro-Glu-Asp-Gly-Gly-Ser-Gly-Ala-Phe-Pro-Pro-Gly-														
16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
His-Phe-Lys-Asp-Pro-Lys-Arg-Leu-Tyr-Cys-Lys-Asn-Gly-Gly-Phe-														
31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Phe-Leu-Arg-Ile-His-Pro-Asp-Gly-Arg-Val-Asp-Gly-Val-Arg-Glu-														
46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Lys-Ser-Asp-Pro-His-Ile-Lys-Leu-Gln-Leu-Gln-Ala-Glu-Glu-Arg-														
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
Gly-Val-Val-Ser-Ile-Lys-Gly-Val-Cys-Ala-Asn-Arg-Tyr-Leu-Ala-														
76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
Met-Lys-Glu-Asp-Gly-Arg-Leu-Leu-Ala-Ser-Lys-Cys-Val-Thr-Asp-														

91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
 Glu-Cys-Phe-Phe-Phe-Glu-Arg-Leu-Glu-Ser-Asn-Asn-Tyr-Asn-Thr-

106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
 Tyr-Arg-Ser-Arg-Lys-Tyr-Ser-Ser-Trp-Tyr-Val-Ala-Leu-Lys-Arg-

121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
 Thr-Gly-Gln-Tyr-Lys-Leu-Gly-Pro-Lys-Thr-Gly-Pro-Gly-Gln-Lys-

136 137 138 139 140 141 142 143 144 145 146
 Ala-Ile-Leu-Phe-Leu-Pro-Met-Ser-Ala-Lys-Ser.

Claim 2 related to a DNA coding for the amino acid residue sequence of a bFGF polypeptide containing a fragment of the 146-amino acid residue sequence of claim 1. Claim 3 related to a DNA encoding fragment (24-120) of bFGF and claim 4 to a DNA encoding an equivalent or an analogue of the 146-amino acid residue sequence of claim 1. Claim 5 recited a particular DNA sequence deduced from the 146-amino acid residue sequence of claim 1. Claims 6 to 12 related to DNA coding for particular analogues of bFGF or modifications of the DNA. Claims 13, 14 to 15 and 16-21 related to an expression vector, transformed microorganisms and recombinant methods for producing a bFGF polypeptide, respectively. Claims 22 and 23 covered bFGF polypeptides having inter alia $\geq 98\%$ purity (on total proteins). Claim 24 related to fragments (24-120) or (20-110) of bFGF. Claims 25 to 33 related to salts, pharmaceutical compositions, first/further therapeutic, diagnostic or in vitro (claim 33) uses of the polypeptides of claims 22, 23 or 24. Claims 1 to 9 for the Contracting State AT were drafted as corresponding process/method claims, whereas claim 10 was directed to the use of the DNA encoding bFGF to produce a bFGF polypeptide.

VII. While accepting priority entitlement (Article 87 EPC), novelty (Article 54 EPC) and sufficiency of disclosure (Article 83 EPC), the Opposition Division came to the conclusion that providing the amino acid sequence of the bovine bFGF purified according to the technique disclosed in document (D2) did not involve an inventive step (Article 56 EPC) and revoked the patent. The decision was taken on the basis the claims of the main request (claims as granted) and those of the auxiliary request, differing therefrom in that the wording "an equivalent or" in claim 4 (all designated Contracting States except AT) and in claim 1 (AT) had been deleted.

VIII. The following documents are cited in the present decision:

- (D2) Gospodarowicz D. et al., Proc. Natl. Acad. Sci. USA, Vol. 81, pages 6963-6967 (1984);
- (D13) Sommer A. et al., Biochem. Biophys. Res. Comm., Vol. 144, No. 2, pages 543-550 (1987);
- (D61) Second Affidavit of Federick Esch dated 19 September 1997;
- (D78) Klagsbrun M. et al., Proc. Natl. Acad. Sci. USA, Vol. 83, pages 2448-2452 (1986);
- (D80) Second Affidavit of P.J. Lowry dated 30 June 1998;
- (D81) Affidavit of J.J. Schumann dated 5 June 1998;
- (D82) Declaration before the USPTO of D.I. Buckley filed 16 December 1985.

IX. The respondent (opponent) withdrew the opposition.

X. In the oral proceedings held on 14 December 2001, the board informed the appellant that a closer examination of the grounds of appeal as they had been received in due time had revealed that all the salient points had been mentioned there. A new citation was provided by the appellant:

(D86) Third Affidavit of Prof. Len Hall dated
13 December 2001.

XI. The submissions by the appellant were essentially as follows:

- Document (D2) represented the closest prior art because it disclosed a three-step purification process for bovine bFGF, the last step of which was a heparin-Sepharose® affinity chromatography (HSAC) step.
- Compared with the purification process disclosed by document (D2), the one according to the patent in suit was a four-step process since the protein product from the HSAC step was pumped onto a Vydac® column for an additional RP-HPLC step which removed the residual 10% contamination. It required inventive skill to obtain a preparation of bovine bFGF sufficiently pure for amino acid sequencing.
- It was impossible to up-scale the process of document (D2) to obtain more bovine bFGF. It was the merit of the invention to have successfully performed an up-scaling to 3 kg bovine pituitaries.
- Sequencing a novel protein having a length of about 150 amino acid residues represented a lengthy task involving months of work and a

plethora of problems which could not reasonably be expected to be overcome by an average protein sequencer.

- Fragmentation of the protein being sequenced was a complex operation conducted in the dark and which moreover occurred with losses. The "protein puzzle" (reassembly of the overlap peptides obtained by fragmentation) could lead to incorrect sequence data.

- There was evidence of failure by three laboratories which attempted to sequence bFGF without complete success between 1985 and 1987. All these groups experienced serious and unsolved difficulties in sequencing bFGF:
 - The Massachusetts General Hospital (MGH) group failed to sequence the tumour angiogenic factor (TAF) from rat chondrosarcoma and human bFGF from SK-HEP1 cell, shown later to have both identical N- and C-terminal sequences as bFGF (see document (D82), paragraph 3 and document (D78)).

 - The opponent (SCIOS) also failed. One ml of 160 µg/ml (10 nmol) was handed over to SCIOS (see Exhibit JS1 to document (D81)). Dr Fiddes ordered 10 pounds pituitaries to purify 800 µg (50 nmol) bovine bFGF. In spite of this abundant starting material, sequencing failed. They incorrectly fused two bFGF fragments together (see page 59 of Dr Abraham's notebook (Exhibit JS6 to document (D81))).

 - The Synergen group (see document (D13)) could also not identify several residues.

XII. The appellant (patentee) requested that the decision under appeal be set aside and that the patent be maintained on the basis of the claims as granted (main request) or the auxiliary request filed in the proceedings before the Opposition Division.

Reasons for the Decision

Admissibility of the appeal

1. The notice of appeal and the appeal fee were submitted within the prescribed time limit. Regarding the grounds of appeal, the board first observes that the appeal would be admissible only to the extent that grounds for the appeal had been received on or before 8 June 1998. On that date, the appellant filed seven different faxes containing evidence, being either affidavits or state of the art documents but containing no grounds. The extent of the appeal therefore depends on the content of the 16 pages faxed and received on 5 June 1998. Since these 16 pages contain at least summary arguments regarding all the grounds indicated in the notice of appeal, and the amended notice of appeal was quite detailed, the appeal is admissible to the full extent intended by the appellant.

Inventive step

2. The only point at issue is the inventive step. The board agrees that the closest prior art is represented by document (D2) because it discloses bovine bFGF (purity of 90%) and a three-step (ammonium sulfate precipitation, ion exchange chromatography on carboxymethyl Sephadex® C-50 and heparin-Sepharose® affinity chromatography (HSAC)) purification process yielding 150 µg (about 10 nmol) bovine bFGF from 1.8 kg

bovine pituitaries. The problem underlying the patent in suit is the provision of a DNA molecule encoding bovine bFGF. This problem is solved by the disclosure of the complete amino acid sequence of bovine bFGF of claim 1, from which a DNA encoding this protein can be deduced according to the genetic code. The synthetic DNA of claim 5 is an example of such a DNA molecule encoding bovine bFGF.

3. The relevant question in relation to inventive step is whether the skilled person would have arrived in an obvious manner at the claimed DNA molecule and would have reasonably expected so to arrive. In the present situation, the board observes that **two** routes were open to skilled person wishing to arrive at a DNA encoding bovine bFGF: (i) providing the amino acid sequence of bovine bFGF and hence deducing/designing therefrom the DNA encoding this protein and (ii) picking up the DNA directly from a cDNA/genomic library.

4. As regards route (ii) above to the claimed DNA, the appellant has argued before the Opposition Division that attempts to isolate a DNA encoding bovine bFGF by library screening with DNA probes designed in the light of the **limited** N-terminal amino acid information available before the priority date of the patent in suit, failed. The board is convinced that it is only with the knowledge of the **full** amino acid sequence of bovine bFGF, as provided by the patent in suit, that it is possible to abandon the G/C rich N-terminal region and select successful probes downstream of the N-terminal 32 amino acid residues (see also document (D61), points 12.3 and 15). In conclusion, route (ii) was not practicable.

- 5. The decisive question thus becomes whether the skilled person starting from the prior art referred to under point 2 above, and based on other relevant prior art knowledge, would have had a reasonable expectation of success in sequencing the bovine bFGF (route (i) above).
- 6. In seeking an answer to the above question, the board observes that three laboratories (termed "SCIOS", "MGH" and "Synergen" below), in addition to that of the patentee attempted to sequence bFGF between 1985 and 1987:

SCIOS

Dr Fiddes of SCIOS (formerly CBI) received 1 ml of 160 µg/ml (10 nmol) pure bovine bFGF (see Exhibit JS1 to document (D81)). Cleavage of the protein occurred with dilute acid (document (D81), paragraph 1.2.1) or CNBr (ibidem, paragraph 1.2.5.2). The fragments were purified by HPLC and sequenced on a gas phase protein sequencer (ibidem, paragraph 1.2.5.2). The "SCIOS" group failed to obtain the correct amino acid sequence of bovine bFGF since upon reassembly of the overlap peptides, they incorrectly fused two bFGF fragments together, as shown by page 59 of Dr Abraham's laboratory notebook (Exhibit JS6 to document (D81)). They identified, inter alia, the sequence:

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41  42  43  44  45  46  47  ....
A   N   R   Y   L   A   M.....
Ala Asn Arg Tyr Leu Ala Met....

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It has turned out in the light of the correct amino acid sequence provided by the patent in suit that the position of this sequence in the bovine bFGF protein was wrong. It starts at position 70.

MGH

According to the legend to Figure 4 on page 1451 of document (D78), the Massachusetts General Hospital ("MGH") group digested 3.2 nmol of the hepatoma-derived growth factor (HDGF) from SK-HEP1 cells (shown in this document to have N- and C-terminals identical to those of human and bovine bFGF) with trypsin and the tryptic peptides were separated by RPLC, yielding 50 pmol of peptide I and 1.3 nmol of peptide II, which were sequenced on an Applied Biosystems sequenator (ibidem, page 2449, r-h column, line 13). Successful sequencing was confined to the above mentioned N- and C-terminal sequences of the protein.

Synergen

According to document (D13), page 545, the "Synergen" group subjected from 100 to 500 pmol of the uncleaved human placental bFGF or of the purified Lys-C-, V8-protease- and submaxillaris-protease-cleaved fragments thereof to sequencing using an Applied Biosystems gas-phase protein sequencer. They were able only to obtain partial protein sequence (ibidem, see page 546: "Residues 18-21, 30-33, 117-120, and 131-135 were not ambiguously identified by protein sequencing and were deduced from the cDNA sequence").

7. Since all three groups belonged to specialized biomedical research facilities, the board is satisfied that they were (at least) of ordinary skill in the art of protein sequencing. All three groups (and the patentee's group) employed state of the art automated gas phase protein sequenators available since 1983 from Applied Biosystems ("ABI"). As regards the state of purity of the starting material, all these groups (and the patentee's group) benefited from the "breakthrough" represented by the HSAC step (see point 2 supra) which

achieved 90% pure bovine bFGF. As for the protein quantities available, these were tiny but substantially in the same order of magnitude for the three groups (and the patentee's group). The limiting step when subjecting to amino acid sequencing tiny quantities of starting product seems to reside in the fragmentation of the protein and the subsequent separation by HPLC of the cleavage products, which may be affected by considerable losses (see point 10 below). In view of this, the conclusion cannot be drawn that these three groups worked under more unfavourable conditions compared with a skilled person (or the patentee's group) faced with the problem of sequencing bFGF at the priority date of the patent in suit.

8. In the board's view, the fact that the "SCIOS", "MGH" and "Synergen" groups all failed to provide the correct or complete amino acid sequence of bFGF truly reflects serious and unsolved difficulties the skilled person might have been confronted with upon sequencing this **specific** bovine bFGF and which required inventive effort to be overcome.

9. One of these problems could lie in selecting the "right" sequencing strategy which plays an important role in the case of an unknown, about 150 amino acid long protein. In addition to N-terminal amino acid information derivable from the intact protein, the latter has to be fragmented by means of one or more of eg endoproteinase Lys-C, V8-protease, submaxillaris-protease, trypsin, chymotrypsin, dilute acid, CNBr, etc, and the overlap peptides must be reassembled for establishing the order of the fragments in the protein. A wrong strategy may lead to an incomplete or incorrect sequence. This view is supported by Figure 1 of document (D13), from which it can be seen that, in spite of the amino acid sequence information derived

from sequencing the intact protein and from the fragments obtained by three cleavages with endoproteinase Lys-C, V8-protease and submaxillaris protease, the reassembled proteolytic fragments do not overlap the whole length of the protein, leaving stretches which have to be determined via the DNA (ibidem, see page 546). The incorrect reassembly of two bFGF fragments noted on page 59 of Dr Abraham's laboratory notebook (see point 6 supra) is a further demonstration that this problem actually turns up.

10. Another problem could arise with the considerable losses occurring during the fragmentation of the protein and subsequent separation of the fragments by HPLC. It can be deduced from Figure 1 of document (D13) that some (short) peptides cannot be recovered. Heavy losses of protein digests are also shown by the legend to Figure 4 (bottom of page 2451 of document (D78)), according to which 3.2 nmol HDGF were digested with trypsin, the tryptic peptides separated by RPLC and only 50 pmol of "HDGF tryptic peptide I" sequenced (in contrast to the 1.3 nmol, ie, a 26-fold quantity of the "HDGF tryptic peptide II"). These facts confirm the appellant's view expressed under paragraph 1.8.2 of document (D80) that digest losses may in certain cases reach 100%.

11. Therefore, the board must conclude that providing the amino acid sequence of bovine bFGF recited in claim 1 and hence deducing/designing therefrom the DNA of claim 1 encoding this protein (route (i) above) involves an inventive step.

This conclusion has to be extended to claims 2 to 12 since they relate to DNAs coding for a fragment of the 146-amino acid residue sequence of claim 1, for fragment (24-120) of the 146-amino acid residue sequence of claim 1, for an equivalent or an analogue

of the 146-amino acid residue sequence of claim 1, or to a particular DNA sequence deduced from the 146-amino acid residue sequence of claim 1 or to a DNA encoding particular analogues of bFGF or cover modifications of the DNA. The above conclusion also applies to claims 13, 14 to 15 and 16 to 21 relating to an expression vector, transformed microorganisms and recombinant methods for producing a bFGF polypeptide, respectively, including or involving the DNA sequence of claim 1.

Claims 22 and 23 cover bFGF polypeptides having inter alia a purity $\geq 98\%$ (on total proteins) while claim 24 relates to fragments 24-120 or 20-110 of bFGF. For any of this claimed subject-matter to be carried out, one must have available either the recombinant route and hence the DNA sequence (or subsequence) of claim 1 or the knowledge of the amino acid sequence 20-120 recited in claim 1. Thus since inventive step can be acknowledged for the provision of both the amino acid and the DNA sequences of claim 1, it can be acknowledged for all these other claims as well. This conclusion also applies to the subject-matter of claims 25 to 33, relating to salts, pharmaceutical compositions, first/further therapeutic or diagnostic and in vitro (claim 33) uses of the polypeptides of claims 22, 23 and 24.

Finally, this conclusion has to be extended to claims 1 to 9 for the Contracting State AT since they are drafted as corresponding process/method claims and to claim 10, directed to the use of the DNA encoding bFGF to produce a bFGF polypeptide.

12. No need arises to consider the auxiliary request.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.
2. The patent is maintained as granted.

The Registrar:

The Chairwoman:

P. Cremona



U. M. Kinkeldey