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D E C I S I O N
of 27 June 1996

Case Number: T 0844/93 - 3.3.4

Application Number: 82304512.5

Publication Number: 0073656

IPC: C12N 15/85

Language of the proceedings: EN

Title of invention:
Preparation of polypeptides in vertebrate cell culture

Patentee:
GENENTECH, INC.

Opponent:
Pasteur Merieux Serum et Vaccins
Institut Pasteur

Headword:
Hepatitis B surface antigen in vertebrate cell culture/GENENTECH

Relevant legal provisions:
EPC Art. 56

Keyword:
"Inventive step (no)"

Decisions cited:
-

Catchword:
-

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0844/93 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 27 June 1996

Appellant: (Proprietor of the patent)
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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 2 August 1993 revoking European patent No. 0 073 656 pursuant to Article 102(1) EPC.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: R. E. Gramaglia
S. C. Perryman

Summary of Facts and Submissions

I. European patent No. 073 656, based on European patent application No. 82 304 512.5 was filed on 26 August 1982.

II. Oppositions were filed by the Respondents (Opponents) requesting revocation of the patent in its entirety on the grounds of lack of novelty and of inventive step (Articles 52, 54, 56 and 100(a) EPC).

III. The Opposition Division revoked the patent. The decision was based on the set of claims as granted and on five sets of claims submitted as auxiliary requests. Claim 1 as granted for all the contracting states except AT read as follows:

"1. A recombinant lytic viral expression vector capable, in a transformant vertebrate cell culture, of expressing a DNA sequence encoding mature hepatitis B surface antigen (HBsAg) lacking any sequence encoding HBsAg precursor sequence, said sequence replacing the coding sequence of a vector viral gene so as to be under the proper control of the promoter of that viral gene heterologous to HBsAg, the translational start codon for the DNA sequence coding for HBsAg being located at substantially the original position occupied by the translational start codon for the viral protein normally controlled by the viral promoter."

IV. The Opposition Division considered that the closest prior art was document

(1) Moriarty et al., Proc. Natl. Acad. Sci. USA, Vol. 78, pages 2606-2610 (1981)

an academic research publication reporting the creation of a recombinant viral expression vector formed by the insertion of the DNA sequence encoding both mature hepatitis B surface antigen (HBsAg) and its precursor sequence, into the viral genome of SV40, which vector was capable of causing expression in a transformant monkey kidney cell culture of the DNA sequence encoding the mature hepatitis B surface antigen (HBsAg) DNA as evidenced by the secretion of 22 nm particles. The information given in document (1) rendered obvious the vector of claim 1 as an alternative to the one specifically disclosed in document (1).

Further documents referred to in the present decision are:

(11) Dubois et al., Proc. Natl. Acad. Sci. USA, Vol. 77, pages 4549-4553 (1980)

(14) Ammerer et al., Recombinant DNA, Proceedings of the Third Cleveland Symposium on Macromolecules (22-26 June 1981), pages 185-197)

(24) Liu et al., DNA, Vol. 1, pages 213-221 (1983)

(25) Hamer et al., Nature, Vol. 281, pages 35-40 (1979)

(S1) Fiers et al., Nature, Vol. 273, pages 113-120 (1978)

(S10) Dreesmann et al., J. Gen. Virol., Vol. 19, pages 129-134 (1973)

V. The Appellant (Patentee) lodged an appeal against this decision. A statement of Grounds of Appeal was submitted on 13 December 1993.

VI. In support of inventive step the Appellant submitted in writing and at the oral proceedings that the viral expression vector of claim 1 was not obvious over the one disclosed by document (1) for two reasons:

- In the construct of document (1), there were pre-sequences, i.e., the hepatitis B virus (hereafter: HBV) DNA insert coded for most of the HBsAg presequence (pre-S) and for the whole S-protein (HBsAg), while the expression vector required by claim 1 had to be devoid of presequences and coded for the mature S-protein only. The skilled person would not have removed the pre-S sequences from the construct of document (1) to arrive at the claimed vector because it was not obvious that HBsAg particles would be formed in mammalian cells using only the DNA sequence coding for the mature S-protein, that is, the mature HBsAg antigen. This was especially true since document (1) suggested a fusion polypeptide that was a C-terminally extended VP-2 protein and document (11) suggested that the HBsAg putative precursor was required for ensuring engagement of the host cell's processing machinery leading to HBsAg particles. In the construct of document (1), the fragment coding for HBV had been inserted at the 3'-end of the SV40 VP-2 DNA and in reading frame therewith, at a HaeII site which was about 90 amino acids downstream from the N-terminus of VP-2. Owing to the presence of the VP-2 start codon and other AUGs, the skilled person had no reason to believe that initiation of translation would have occurred at the start codon of the mature S-protein. Rather, document (1) would have led to the idea that the particles must have been constructed from a fusion protein or from some other longer precursor. The fact that the detected expression product was in the form of

secreted and glycosylated particles suggested that the precursor product engaged the cell's secretory machinery, was glycosylated and further processed to remove the polypeptide N-terminal to the mature S-protein and was assembled into particles. The SDS gel of document (1) (see Figure 4 on page 2609) showed an additional band P5 of higher molecular weight that could have been taken by the skilled person for an imperfectly processed longer precursor. Figure 4C, lane 12 also showed a band labelled VP1, however, this band was nothing else than the fusion protein P5. In view of this it was unexpected that inserting only the DNA fragment coding for the mature S-protein without any pre-sequences would have resulted in the production of HBsAg particles.

- In the vector of document (1), the DNA coding for the S-protein (HBsAg) was located within the DNA coding for the viral protein VP-2, while in the vector of present claim 1, it was required that the translational start codon of the DNA sequence coding the mature S-protein (HBsAg) be located at substantially the same position as the ATG start codon of the DNA coding for the viral protein normally controlled by the viral promoter. This expedient rendered possible an expression level comparable with that of the native protein, and some thirty times better than for the vector of document (1). In the prior art vectors such as the one disclosed by document (1), the DNA coding for the protein of interest was inserted anywhere in the vector and the expression yields were poor. No effort had been made in the prior art to tailor the promoter-gene distance in the manner of the patent in suit because there was no expectation of any particular benefit from doing so. According to the patent in suit the ATG start codon of the DNA

sequence coding for HBsAg is located at the original position occupied by the translational start codon of the DNA coding for the viral protein normally controlled by the viral promoter. Such vector system rendered possible higher expression yields, as compared with the prior art vectors' expression yields. This was shown by the 3.8 µg HBsAg/$1x10^6$ cells (patent in suit, column 12, line 28) versus 2.5 µg HBsAg/$2x10^7$ cells of document (1) (see page 2608, l.h. column, lines 11-12). There was thus in the prior art neither the suggestion that it would have been useful to adjust in said vector the distance between the promoter and the DNA coding for the protein to be expressed, nor was there any established opinion that the ATG start codon of the DNA coding for the protein to be expressed should be located in the same position as the ATG start codon for the native protein.

VII. The Respondents essentially submitted the following argument in writing and at the oral proceedings:

- Much was already known about the SV40 vector used in document (1), and the first thought of the skilled person seeking to improve the strategy used in document (1), would be to try and optimize the conditions by including only the sequence coding for the desired end product, and by imitating the natural conditions for expression of the proteins coded for normally by SV40 as closely as possible, by locating the translational start codon for the DNA sequence coding for HBsAg at substantially the original position occupied by the translational start codon for a protein normally coded for by SV40. The skilled person would thus as first thought do what claim 1 of the main request was directed to. Further as the VP1

protein is the viral protein most expressed by natural SV40, the position occupied by the start codon for VP1 would be the first one that the skilled person would try, and this is what claim 1 of the auxiliary requests 3 to 5 is directed to.

- Although some authors had considered that the HBsAg pre-S sequences might play a role in the expression of HBsAg particles, there were in the prior art documents no statement that these presequences were necessary for expressing HBsAg particles. Document (1) failed to present any evidence of a fusion protein. The additional band P5 of higher molecular weight shown in Figure 4 on page 2609 of document (1) which, in the Appellant's view, could have been taken by the skilled person for an imperfectly processed longer precursor, was nothing else than an artifact produced by the aggregation of proteins P1 and P2.

- That there was higher expression of cells transfected with the construct of claim 1 of the patent in suit in comparison with the vector of document (1) was contested, because like was not being compared to like.

- Document (14) taught on page 189 that it was desirable to join the coding region being transcribed to a promoter in such a way that the protein made has no N-terminal extension beyond that of the natural protein.

VII. In the communication accompanying the summons to oral proceedings and at the oral proceedings on 27 June 1996 the Board had expressed doubts as to whether certain claims of the then pending requests would satisfy the requirements of Articles 54 and 56 EPC. In response, the Appellant filed at the oral proceedings a new main

request and new first to fifth subsidiary requests. Claim 1 of the main and of the first and second subsidiary requests was the same as Claim 1 as granted. Claim 1 of the third, fourth and fifth subsidiary requests read as follows, changes compared to claim 1 as granted being by underlining or striking out respectively:

"1. A recombinant lytic SV40 viral expression vector capable, in a transformant vertebrate cell culture, of expressing a DNA sequence encoding mature hepatitis B surface antigen (HBsAg) lacking any sequence encoding HBsAg precursor sequence, said sequence replacing the coding sequence of ~~a vector viral~~ the VP1 gene so as to be under the proper control of the promoter of that ~~viral~~ VP1 gene heterologous to HBsAg, the translational start codon for the DNA sequence coding for HBsAg being located at substantially the original position occupied by the translational start codon for the ~~viral~~ VP1 protein normally controlled by the viral promoter."

IX. The Appellant (Patentee) requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request or one of the first to fifth subsidiary request filed at the oral proceedings on 27 June 1996.

The Respondents (Opponents) requested that the appeal be dismissed.

Reasons for the Decision

Main request Claim 1

Articles 123(2) and (3) EPC

1. Claim 1 of the main request corresponds to claim 1 as granted, and no question under Article 123 EPC arises.

Novelty

2. The novelty of claim 1 has never been questioned by the Respondents. Since the Board is in agreement with this conclusion, there is no need for further reasoning on this point.

Inventive step

Closest prior art

3. In relation to the viral lytic expression vector of claim 1, document (1) is regarded by the Parties as the closest prior art document and the Board agrees as well. In this academic work, the authors rely on the regulatory signals of SV40 without refinement or fine tuning to investigate whether expression and secretion of 22 nm HBsAg particles is possible. The construct of document (1) thus differs from the one of claim 1 of the patent in suit by two features. The first one is the presence in the expression vector of document (1) of a 1350-bp insert of hepatitis B virus DNA which codes for most of the HBsAg presequence (pre-S) and for the whole S-protein, while the expression vector of claim 1 is devoid of presequences and codes for the mature S-protein only. The second difference is the position of the HBV DNA insert, which in the vector of document (1) is located within the DNA coding for the

coding for the viral protein VP-2, i.e, the ATG start codon for the mature S-protein is preceded by a portion of DNA sequence coding for SV40 VP-2, some pBR322 DNA and part of the pre-S DNA, while in the vector of present claim 1 the translational start codon of the DNA sequence coding the mature S-protein is located at substantially the same position as the ATG start codon of the DNA coding for the viral protein normally controlled by the viral promoter.

Problem to be solved and its solution

4. In the light of document (1), the technical problem to be solved by the patent in suit can be seen in the fine tuning of the construct of document (1) so as to provide new tools for expression in a vertebrate cell culture of a DNA sequence encoding mature HBsAg so as to obtain mature HBsAg with a view to its use in the preparation of a vaccine. The solution as claimed in Claim 1 of the main request is a recombinant viral lytic vector wherein the sequence encoding for HBsAg lacks any sequence encoding HBsAg precursor sequence, is under the control of a viral promoter, the ATG start codon thereof is located at substantially the same position as the translational start codon of the DNA coding for the viral protein normally controlled by the viral promoter. In view of the Example, the Board is satisfied that this problem has been solved.

5. The first question for the Board to decide is what would a skilled person do in attempting to solve the above stated problem starting from document (1). The Board agrees with the Respondents' view that the first thought of the skilled person seeking to improve the strategy used in document (1), would be to try and optimize the conditions by including only the sequence coding for the desired end product, and by imitating the natural conditions for expression of the proteins

coded for normally by SV40 as closely as possible, by locating the translational start codon for the DNA sequence coding for HBsAg at substantially the original position occupied by the translational start codon for a protein normally coded for by SV40.

Expression of the HBsAg precursor sequence would be avoided, as it was neither required nor desired for use in a vaccine. The skilled person seeking to optimize the system would thus seek to omit this sequence coding for this precursor. This appears to require only routine work, and the contrary has not been argued by the appellant.

Further, it was known that VP-1 protein was the major capsid protein of SV40 (see document (S1) page 113, left-hand column, lines 6 to 7 from the bottom) and that it was synthesized in amounts larger than that of the minor structural elements VP-2 and VP-3. The skilled person wishing to obtain high expression levels under the control of a viral promoter as first choice would opt for replacing the DNA encoding for the VP-1 capsidal protein with the DNA coding for the protein being highly expressed rather than opt for the replacement of DNAs coding for poorly expressed viral genes such as VP-2 and VP-3.

The Appellant's proposition that the skilled person would not have tailored its construct in the manner recited in claim 1, namely with the ATG for the protein being expressed at substantially the same location of the ATG for VP-1, because there was no expectation of any particular benefit, cannot be accepted by the Board. The skilled person was aware of the fact that nature provided a high level of expression of VP-1. Therefore, if one wished an expression comparable with that of the viral protein VP-1 in nature, the first solution that came to the skilled person's mind to

improve on document (1) would be to assemble the construct in such a manner as to mimic the natural situation as closely as possible. The Board sees no technical obstacle to this obvious approach being put into practice, nor was it argued by the Appellant that such obstacle existed.

A more thorough approach to optimization of expression level would have been to screen a series of constructs with variable distances between the promoter and the ATG start codon and with variable DNA sequences preceding the latter, in relation the original position occupied by the translational start codon for both VP1 and VP2 and possibly other SV40 proteins. This more thorough approach would always involve testing the ATG start codon for HBsAg at substantially the original position occupied by the translational start codon for the VP1 protein.

6. Further the Board has to decide whether the skilled person would have considered carrying out the first line of thought stated above in point 5 with a reasonable expectation of success. That the procedure of document (1) with a fairly rough and ready insertion of DNA coding for HBsAg and some of its precursor sequence, lead to successful expression of HBsAg might well have been surprising to the skilled person. However given this critical information, in the Board's judgement, the skilled person would be confident that with fine tuning he could get an improved vector, and would therefore embark on the course outlined in point 5 with a reasonable expectation of success.

7. The Appellant's first line of argument to counter the view that it was obvious to tailor the vector of document (1) in such a way as to include only the sequence coding for the S-protein devoid of any pre-S sequence, was that document (1) suggested a fusion

polypeptide that was a C-terminally extended VP-2 protein and document (11) suggested that HBsAg putative precursor was required for the expression of HBsAg particles. The Board disagrees. A comment on the vector of document (1) can be found in the later document (24), whose authors are also inventors of the patent in suit. This gives the Board an opportunity to know how a skilled person viewed the SV40 vector of document (1) at the priority date of the patent in suit. The authors of document (24) do not share the Appellant's view that the construct of document (1) encodes a fusion protein but merely state (see page 220, paragraph bridging l-h and r-h columns) that due to the nature of this construct, **no firm conclusions** could be drawn regarding the involvement of potential signal peptides preceding the coding region of the mature HBsAg.

8. The Board comes to the same conclusion as the authors of document (24). It was indeed impossible for a skilled person to establish with certainty whether in the construct of document (1), the insert coding for HBsAg was in phase with any of the viral ATG start codons preceding it and thus whether a fusion protein was expressed. The authors of document (1) never say that this happened and they do not pay attention to this aspect, but merely focus on the regulatory signals (see page 2610, lines 6-10), namely the SV40 late promoter, the late 19S mRNA splice junction and the late region polyadenylation signal. These elements were fundamental to the construct of document (1), not the VP-1, VP-2, or VP-3 or any other start codon possibly in phase with the DNA coding for the mature S-protein. This view is supported by how the authors of document (1) designed their construct, namely by assembling a linear vector and inserting it as such in monkey cells, whereby the cell machinery circularized the vector. Cyclisation *in vivo* had already been

applied in the construction of the SV40 vector of document (25) comprising the β^{maj}-globin gene (see last step of the scheme on page 36). However, this cyclisation was frequently accompanied by deletions around the junctions between the viral and foreign DNAs (see page 37, 1-h column, lines 7-11). Dr D.H. Hamer, who is co-author of both documents (1) and the earlier document (25) was fully aware of this highly probable phenomenon when he designed, together with his co-workers, the vector of document (1). In these circumstances, the probability that some VP-1/VP-2/VP-3 or pBR322 ATG start codon could have occurred in phase with the S-protein start codon, and that such ATG start signal could have been followed by sense triplets, is small. If the authors of document (1) or any other skilled person wanted a fusion protein, the route disclosed by document (1) involving the cyclisation in vivo with a highly probability of random, and thus uncontrollable deletions, was far from recommendable. A technique involving synthetic oligonucleotide linkers, such as the one used in the patent in suit, would have been more suited to obtaining a fusion protein because this method, unlike the one disclosed by document (1) was controllable. While the skilled person would have been aware of the possibility that, owing to the presence of the VP-2 start codon and other ATGs, initiation of translation might have occurred at start codons other than the start codon for the mature S-protein, this possibility was very remote because these start codons needed to be followed by sense triplets, i.e. to be in reading frame, and assembling a vector with a technique implying a random deletion is not the right way to ensure that this condition be fulfilled. Therefore, the Board concludes that the skilled person reading document (1) would not be under the impression that a fusion protein had been expressed by cells transfected with the SV40-HBV vector.

9. As to the Appellant's argument that the SDS gels of document (1) (see Figure 4 on page 2609) showed an additional band P5 of higher molecular weight that could have been taken by the skilled person for an imperfectly processed longer precursor, the Board observes that said P5 band appears to be a mere artifact due to oxidation since it does not turn up when HBsAg is biosynthetically labelled (see document (1), page 2609, bottom of 1-h column). The Appellant's argument that P5 cannot be a -S-S- bridged dimer because 2% mercaptoethanol is added before electrophoresis (see legend to Figure 4) occurs, is also not convincing. Document (S10)(see page 129) shows that -S-S- bridged polymers of HBsAg are resistant to reduction and need two hours with 0.1M dithiothreitol (DTT) for them to be split. Since 2% mercaptoethanol corresponds to 0.13 M, and bearing in mind that one molecule of DTT comprises 2 -SH's while that of mercaptoethanol has only one -SH (thus 0.1 M DTT are more reducing than 2% mercaptoethanol), the Board finds it reasonable to believe that the conditions of Figure 4 (comprising no two hour incubation) would not split the dimer.

10. Also the Appellant's view that the band labelled VP-1 in lane 12 of Figure 4 C is nothing else than P5 (see point 9 supra) is inconsistent with the presence of the same VP-1 band in lane 11 relating to wild type SV40 with no HBsAg DNA sequences. The Respondents' explanation that this VP1 band turns up because the anti-ad antiserum is capable of also immunoprecipitating VP-1, is more credible to the Board. The Appellant argued that if the anti-ad antiserum were actually capable of binding to VP-1, then a VP-1 band would have to turn up in lane 2 as well, but this is not the case. However it should be noted that lane 2 only relates to fractions 15-17 of the sucrose gradient (see legends to Figures 3, 4 and

to Table 1) while lanes 11 and 12 from experiment B relate to the total medium. It is thus not surprising that VP-1 is present in lane 12 but not in lane 2. As to the hypothesis put forward by the Appellant that the presence of the weaker VP-1 band in lane 11 might represent a leakage from the adjacent lane, this is also not credible since it would imply a selective leakage involving VP-1 only but not the other bands P1 and P2 in the same lane.

11. The appellant's second line of argument in support of an inventive step, namely that the higher expression of cells transfected by the vector of the patent in suit over the one of document (1) is something quite unexpected, and therefore needed inventive ingenuity appears not to be consistent with what was already known about SV40. (See discussion above in point 5). The first thought of the skilled person wishing to obtain high expression levels under the control of a viral promoter would be to opt for replacing the DNA encoding for the VP-1 capsidal protein with the DNA coding for the HBsAg. There would be no preference for the start codon position of poorly expressed viral genes such as VP-2 and VP-3.

12. For these reasons the Board concludes that claim 1 of the main request does not fulfil the requirements of Article 56 EPC, and that the main request must be rejected.

First and second subsidiary requests

13. Claim 1 of these requests is the same as Claim 1 of the main request, which claim has been found by the Board not to comply with the requirements of Article 56 EPC. These requests also must therefore be rejected.

Third subsidiary request claim 1

Articles 123(2) and (3) EPC

14. In claim 1 of the third subsidiary request, the viral vectors encompassed by claim 1 of the main request have been restricted to the SV40 (simian virus 40) vector, and the viral protein encoded by the viral vectors has been specified as VP1. These amendments restricting the scope of granted claim 1 find a basis on page 13, line 23 to page 22, line 13 of the original description. This amended claim thus meets the requirements of Article 123(2) and (3) EPC.

Inventive step

15. The novelty of claim 1 of the third subsidiary request has not been challenged. As for claim 1 of the main request, document (1) is the closest state of the art, and the problem to be solved is again that formulated in point 4. Document (1) already uses the SV40 vector, and as discussed at point 5 above, the first thought of the skilled person would be put the start codon for the DNA sequence coding for HBsAg at substantially the original position occupied by the translational start codon for the VP1 protein normally controlled by the viral protein. The Board thus concludes that claim 1 of this request is also obvious, and accordingly the third subsidiary request must be refused.

Fourth and fifth subsidiary requests

16. Claim 1 of each of these requests is the same as claim 1 of the third subsidiary request held by the Board not to comply with the requirements of Article 56 EPC. These requests too must therefore be refused.

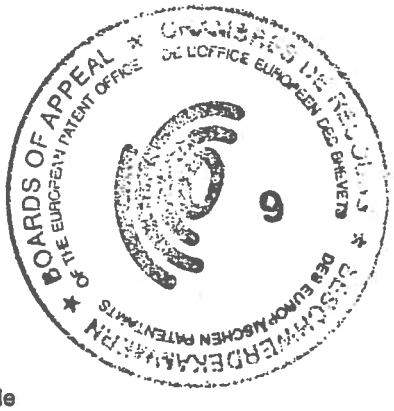
Order

For these reasons it is decided that:

The Appeal is dismissed.

The Registrar:

A. Townend



The Chairwoman:

U. M. Kinkeldey

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