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**D E C I S I O N**  
**of 6 November 2003**

**Case Number:** T 1100/01 - 3.3.8

**Application Number:** 92901535.2

**Publication Number:** 0574402

**IPC:** C12N 15/57

**Language of the proceedings:** EN

**Title of invention:**

Expression of PACE in host cells and methods of use thereof

**Patentees:**

GENETICS INSTITUTE, INC., et al

**Opponent:**

Baxter Aktiengesellschaft

**Headword:**

Truncated transmembrane PACE/GENETICS

**Relevant legal provisions:**

EPC Art. 113(1), 114(1), 123(2)  
EPC R. 67

**Keyword:**

"Main request - added subject-matter (no)"  
"Right to be heard (yes)"  
"Procedural violation (no)"  
"Reimbursement of appeal fee (no)"

**Decisions cited:**

T 0012/81, T 0181/82, T 0007/86, T 0694/92, T 0345/01,  
G 0009/91, G 0010/91, G 0002/98

**Catchword:**

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Case Number: T 1100/01 - 3.3.8

**D E C I S I O N**  
of the Technical Board of Appeal 3.3.8  
of 6 November 2003

**Appellants:** GENETICS INSTITUTE, INC. et al.  
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**Decision under appeal:** Decision of the Opposition Division of the  
European Patent Office posted 17 August 2001  
revoking European patent No. 0574402 pursuant  
to Article 102(1) EPC.

**Composition of the Board:**

**Chairman:** L. Galligani  
**Members:** P. Julia  
S. C. Perryman

## Summary of Facts and Submissions

- I. An appeal was lodged by the patent proprietors (appellants) against the decision of the opposition division, whereby the European patent No. 0 574 402 was revoked. Basis for the revocation were a main request and auxiliary requests A, B and C, which were not considered to comply with Articles 123(2),(3) and 84 EPC, and auxiliary request D, which was considered to comprise added subject-matter (Article 123(2) EPC).
- II. Claim 1 of auxiliary request D before the opposition division read as follows:

"1. A host cell comprising

a recombinant DNA sequence encoding the mammalian paired basic amino acid converting enzyme PACE lacking a transmembrane domain, operably linked to a heterologous expression control sequence permitting expression of said PACE; and a polynucleotide encoding a precursor polypeptide, wherein the precursor polypeptide is a substrate for the encoded PACE which is operably linked to a heterologous expression control sequence permitting expression of the protein product of the precursor polynucleotide by the host cell."

Claims 2 and 3 concerned specific embodiments of the host cell of claim 1, claims 4 and 5 were directed to the corresponding expression vectors. Claims 6 to 8 concerned a method of increasing the yield of a biologically active protein.

III. Claims 1, 3, 5 and 7 as originally filed read as follows:

"1. A host cell comprising a recombinant polynucleotide encoding PACE, wherein the cell is capable of expressing PACE."

"3. The host cell according to claim 1, wherein the encoded PACE lacks a transmembrane domain."

"5. The host cell according to claim 1 further comprising a polynucleotide encoding a precursor polypeptide, wherein the precursor polypeptide is a substrate for the encoded PACE, and wherein the cell is capable of expressing the polynucleotides encoding PACE and the heterologous polypeptide."

"7. The host cell according to claim 5 wherein the encoded PACE and the encoded heterologous precursor polypeptide, when expressed, are secreted into extracellular medium."

Claims 2, 4, 6 and 8 to 19 referred to further embodiments of said host cell, whereas claims 20 to 33 related to a recombinant expression vector suitable for expression in a selected host cell comprising a polynucleotide sequence encoding PACE alone or with another polynucleotide sequence encoding the precursor polypeptide. Claims 34 to 52 related to different methods, such as methods for producing recombinant PACE (claims 34 to 39), methods for producing a desired mature polypeptide (claims 40 to 42) and methods of increasing the yield of a biologically active protein (claims 43 to 52).

- IV. In the statement of grounds of appeal, the appellants discussed only auxiliary request D, which in their view did not contravene Article 123(2) EPC, and a procedural violation allegedly committed by the opposition division.
- V. The opponent (respondent) filed observations in reply to the statements of grounds of appeal.
- VI. The board issued a communication under Article 11(2) of the rules of procedure of the Boards of Appeal indicating its preliminary view on the alleged procedural violation and the compliance of auxiliary request D with Article 123(2) EPC.
- VII. In reply to the board's communication, the appellants filed a set of claims for ES and GR of auxiliary request D.
- VIII. Oral proceedings were held on 6 November 2003. During the oral proceedings the appellants filed a new auxiliary request D for all the designated contracting states except ES and GR which comprised 7 claims and differed from the request D not allowed by the opposition division only in that claim 2 was deleted with consequent revision of the numbering and back-references. An auxiliary request D for ES and GR comprising 7 claims in the form of method claims was also filed.
- IX. The appellants' submissions in writing and during the oral proceedings, insofar as they are relevant to the present decision, can be summarized as follows:

*Procedural violation*

The feature of the claims alleged to be added subject-matter was already present in the granted claims. However, Article 100(c) EPC was not pleaded as a ground of opposition within the statutory opposition term (Article 99(1) EPC). This ground of opposition was raised for the first time during the oral proceedings and it was not clearly pleaded in any of the opponent's written submissions. In these submissions, only the addition of the word "functional" had been objected under Article 100(c) EPC. The patentees were given a strictly limited and insufficient period of time for a proper consideration of the fresh ground of opposition and they were denied the opportunity to respond adequately by refusing the request for an adjournment of the proceedings so as to permit a technical expert to be consulted. Thus, the opposition division committed a procedural violation which justified the reimbursement of the appeal fee.

*The issue under Article 123(2) EPC: added subject-matter*

The term PACE in the application as filed was a generic term and included not only the full-length PACE but (truncated) fragments and analogues thereof that retained the biological activity of full-length PACE as well. A PACE lacking its transmembrane domain was expressly taught as a preferred PACE fragment and repeatedly mentioned in the description as filed. The production of a PACE fragment devoid of transmembrane domain was shown in example 7 and it was explicitly

said to be preferred for the co-culturing method. The co-culturing method (DNA sequences encoding PACE and its substrate were introduced into and expressed in different host cells) was disclosed as a simple alternative to the co-expression method (DNA sequences were introduced into and expressed in the same host cell). Whereas in the co-expression method a PACE lacking the transmembrane domain was only optional, in the co-culturing method this truncated PACE was necessary for the interaction of both PACE and the secreted substrate - a precursor polypeptide - to take place in the same medium. The co-expression of PACE and its substrate was taught to be one of the principal methods for achieving the benefits of the invention and the combination of claims 5 and 7 as filed included the co-expression of PACE lacking the transmembrane domain and of its substrate. Thus, the co-expression - in a single host cell - of both precursor polypeptide (PACE substrate) and PACE lacking the transmembrane domain was directly and unambiguously derivable from the disclosure of the application taken as a whole and there was nothing in the application as filed that pointed away from the use of PACE lacking a transmembrane domain in the co-expression method.

In order to assess the presence of added subject-matter, the whole disclosure had to be taken into account as well as the information directly and unambiguously derivable from the application as filed. According to the established case law of the Boards of Appeal, specific embodiments - in particular, similar exemplary embodiments with comparable elements - could be interchanged where it was technically appropriate to do so, there was nothing in the application as filed

that pointed away from an interchange of full-length PACE and a PACE lacking the transmembrane domain and the resulting combination - in the co-expression method - provided a solution to the problem addressed by the application. In the present case, the application did not disclose a long list of possible PACE fragments, variants or analogues thereof but only a very small number of specific PACE products which were to be used in a very small number of possible methods.

- X. The respondent's submissions in writing and during the oral proceedings, insofar as they are relevant to the present decision, can be summarized as follows:

*Procedural violation*

The ground of opposition was not raised for the first time at the oral proceedings. In the written submissions, it had already been indicated that soluble PACE or PACE lacking a transmembrane domain was not mentioned in the context of co-expression. In the claims as granted, this feature was present only as an alternative with co-expression from a polynucleotide encoding full-length PACE and thus, the amendment carried out in auxiliary request D redefined the invention. Regardless of whether or at what stage objections were raised, the opposition division had to consider whether the amended claims satisfied the requirements of the EPC and, in particular the ones of Article 123(2) EPC. During the oral proceedings, the opposition division offered the patentees further time for considering the objection under Article 123(2) EPC. However, the patentees did not take advantage of it. It was the patentees' choice not to bring a technical



expert at the oral proceedings and there had been no reason to adjourn the proceedings to consult a technical expert.

*The issue under Article 123(2) EPC: added subject-matter*

The application as filed disclosed three different embodiments: (i) a host cell producing recombinant PACE (with or without transmembrane domain), which could then subsequently be used - outside the host cell - as a reagent to cleave the precursor polypeptide (reagent embodiment), (ii) a cell culture producing both PACE and precursor polypeptide in the same cells so that PACE acted on the precursor polypeptide inside the host cell (co-expression embodiment) and (iii) a cell culture with two cell populations, one producing PACE and the other producing precursor polypeptide so that secreted PACE acted on secreted precursor polypeptide - outside the cell - in the shared culture medium (co-culturing embodiment). A PACE lacking the transmembrane domain was said to be preferred only for certain embodiments but not applicable to all of them. The application as filed referred to the importance of the transmembrane domain for cellular location and there was no mention of co-expression of PACE lacking a transmembrane domain and a precursor polypeptide within the same host cell. None of the references to co-expression contemplated the use of a PACE lacking a transmembrane domain. In contrast, PACE lacking a transmembrane domain was said to be preferred and specifically relevant for expression in separate host cells (co-culturing). Thus, the application as filed taught away from the use of PACE lacking a

transmembrane domain in the co-expression embodiment, such a use was not in line with the teaching of the application as a whole.

Example 7 only taught the construction of an expression vector containing a DNA sequence encoding PACE lacking a transmembrane domain but there was no suggestion of using such an expression vector for co-expression in a single host cell. Claim 3 as filed was directed to a host cell comprising a polynucleotide encoding a PACE lacking a transmembrane domain. However, neither claim 5 nor claim 7 as filed were dependent on claim 3. The production of secreted PACE - the subject-matter of claim 7 or the combination of claims 5 and 7 as filed - could well be achieved with full-length PACE by autoproteolysis - due to a large overproduction of PACE in transfected COS-1 cells - or by co-expression with a vWF-producing CHO cell line as shown, respectively, in examples 2 and 5. Thus, claims 5 and 7 as filed did not provide a basis for co-expression in the same host cell of a PACE lacking a transmembrane domain and of a precursor polypeptide. There was neither an explicit disclosure nor an implicit teaching in the application taken as a whole for the co-expression in the same host cell of a recombinant DNA sequence encoding PACE lacking a transmembrane domain and of a polynucleotide encoding a precursor polypeptide. Furthermore, nothing indicated that such co-expression system was advantageous or that it provided a solution to any technical problem.

The generic term PACE encompassed a large number of possible fragments, variants or analogues of the full-length PACE. A PACE lacking a transmembrane domain was

only one out of several possible PACE fragments explicitly mentioned in the application as filed. In accordance with the established jurisprudence of the Boards of Appeal, the combination of elements from two or more lists or else from several possible options - a particular PACE fragment (PACE lacking transmembrane domain) with a particular method of exposing the PACE to a precursor polypeptide (co-expression) from the many methods discussed - led to added subject-matter, representing an individualized form, selection or combination that was not disclosed as such in the application as filed. The claimed subject-matter was an artificial combination - in the sense that the three embodiments were not similar and there were pointers to the contrary in relation to combining features - which could not have been contemplated by the skilled person reading the application as filed and which did not solve the problem addressed by the application. Explicit reference was made to decisions T 12/81 of 9 February 1982 (OJ EPO 1982, 296), T 181/82 of 28 February 1984 (OJ EPO 1984, 401), T 7/86 of 16 September 1987 (OJ EPO 1988, 381) and T 694/92 of 8 May 1996 (OJ EPO 1997, 408; in paragraph 24 of the Reasons) of the Boards of Appeal in support of this line of argumentation.

- XI. The appellants (patentees) requested that the decision under appeal be set aside and that the case be remitted to the first instance for further prosecution on the basis of the sets of claims submitted at the oral proceedings on 6 November 2003, and that the appeal fee be reimbursed.

XIII. The respondent (opponent) requested that the appeal be dismissed.

### **Reasons for the Decision**

1. The board notes that Article 100(c) EPC (Article 123(2) EPC) was not mentioned in the original grounds of opposition. However, according to the decisions of the Enlarged Board of Appeal G 9/91 and G 10/91 of 31 March 1993 (OJ EPO 1993, pages 408 and page 420, respectively), an Opposition Division may, in application of Article 114(1) EPC, of its own motion raise a ground for opposition not covered by the statement pursuant to Rule 55(c) EPC or consider such ground raised by the opponent after the expiry of the time limit laid down in Article 99(1) EPC but only in exceptional cases where, *prima facie*, there are clear reasons to believe that such grounds are relevant and would in whole or in part prejudice the maintenance of the European patent (cf G 9/91, point 16 of the Reasons of the Decision). This is evidently the situation of the present case, where the Opposition Division considered the ground of opposition under Article 100(c) EPC to be so relevant as to revoke the patent. Thus, no criticism can be levelled at the opposition division for using its discretion to introduce the said belated ground of opposition.

*The issue under Article 123(2) EPC: added subject-matter*

2. The subject-matter of the claims now before the board relates to the co-expression in a host cell of a

recombinant DNA sequence encoding the mammalian paired basic amino acid converting enzyme (PACE) lacking a transmembrane domain and a polynucleotide encoding a precursor polypeptide, which is a substrate for the encoded PACE (cf Section VIII *supra*). The opposition division decided that a PACE lacking the transmembrane domain was not disclosed in the application as originally filed in the context of co-expression with a precursor polypeptide.

3. In accordance with the established case law of the Boards of Appeal, the content of an application as filed comprises the whole of the technical information which is directly and unambiguously derivable therefrom including information which is implicitly apparent - using common general knowledge - to a person skilled in the art, when reading the application. This "whole content approach" or disclosure test is essentially the same used for judging novelty and entitlement to priority and excludes eg the making of arbitrary links between features from different parts of the application which links are not as such derivable from the entirety of the disclosure (cf G 2/98 of 31 May 2001, OJ EPO October 2001, pages 413 to 433 and T 345/01 of 14 February 2003 point 1 of the Reasons of the Decision).
4. The disclosure of the application as a whole concerns the production of recombinant PACE as well as methods for using this PACE, in particular for increasing or enhancing the processing of selected precursor polypeptides - substrates of said PACE - into the corresponding mature polypeptides. In order to carry out these methods, both the PACE and the precursor

polypeptide have to be brought into contact so that the latter can be efficiently processed - cleaved - into its mature form by PACE. This contact is an essential technical feature common to all disclosed methods.

5. The application as filed refers to three expression systems for the production of recombinant PACE, namely mammalian cells, microorganism cells - including yeast and bacteria - and insect cells (cf page 23 to page 52). Reference is made in all of them to PACE in general and to fragments thereof (cf *inter alia* page 23, line 11, page 30, lines 5 to 11, page 32, line 10, page 37, line 19) as well as to different means for achieving their expression, such as expression vectors, promoters, terminator sequences, etc. and, in particular, leader sequences for secretion into the growth media (cf *inter alia* page 33, lines 3 to 35 and page 41, lines 3 to 29). They are all defined as being suitable for said expression and, the fact that some of them are characterized as preferred ones, does not mean that the others are to be disregarded.
  
6. Similarly, the application as filed discloses several methods of using recombinant PACE for enhancing the processing of selected precursor polypeptides into the corresponding mature polypeptides. In particular, recombinant PACE can be produced by transformed host cells and subsequently used as an added reagent to the precursor polypeptide (reagent embodiment). Recombinant PACE can be localized either in the host cell membrane or else as soluble, secreted recombinant PACE. In the latter case, the precursor polypeptide can also be expressed and secreted by host cells in a shared culture medium so that contact between PACE and the

precursor polypeptide takes place in a straightforward manner outside the host cells (co-culturing embodiment) (cf page 22, lines 6 to 8). In another method, recombinant PACE and precursor polypeptide can be produced and expressed in the same host cell (co-expression embodiment). In this case, the essential contact between the PACE and its substrate can be either inside the host cell - when there is no secretion - or else outside the host cell - with secretion of both products. The secretion of both the PACE and the precursor polypeptide is explicitly claimed in the combination of claims 5 and 7 as originally filed, this specific embodiment being particularly important for precursor polypeptides which are known to be naturally secreted polypeptides. Claim 5 as filed defines PACE as a similarly generic term as in claim 1 as filed, which term embraces both full-length PACE and PACE lacking a transmembrane domain, as also shown by dependent claim 3 as filed.

7. The application as filed clearly states that the function of the PACE transmembrane domain is to localize the full-length PACE into the host cell membrane (cf page 20, lines 3 to 5) and thus, impair the secretion of said full-length PACE outside the host cell. On the other hand, a PACE lacking the transmembrane domain is clearly identified as being secreted outside the host cell (cf *inter alia* page 20, lines 25 to 29 and page 22, lines 7 to 8). Thus, the board understands that the whole content of the application as filed directly and unambiguously teaches the skilled person to use such a PACE lacking the transmembrane domain in the co-expression system of claim 7 as filed.

8. The board cannot follow respondent's arguments that in view of the results shown in examples 2 and 5, the skilled person would understand the combination of claims 5 and 7 as relating exclusively to full-length PACE.

8.1 Example 2 concerns the expression of full-length PACE in mammalian COS-1 cells and discloses the presence of secreted low molecular weight (75kDa) PACE species, which differ in apparent size from the intracellular (90kDa) PACE species. These secreted PACE species are said to be the result of autoproteolysis of the full-length PACE due to a large overproduction of PACE in transfected COS-1 cells. Example 5, which relates to the expression of full-length (90-100kDa) PACE in CHO cells with and without co-expression of vWF, also discloses an apparent secretion of smaller (75-80kDa) PACE species in the conditioned medium.

8.2 However, the board notes that in example 2 the relative quantity of secreted PACE observed in the medium is 5 to 10 fold less than that detected in the cell lysate or remaining inside the cell at 12 hour chase period (cf page 58, lines 3 to 27). This secreted PACE is also identified as being a PACE probably missing its transmembrane domain and thus, fully in agreement with the disclosed function of the transmembrane domain. Moreover, in example 5 the smaller, secreted PACE is detected in the conditioned medium at 12 and 18 hours, whereas the secreted vWF is said to be completely processed to mature vWF at already 12 hours (cf page 64, line 28 to page 65, line 18).



- 8.3 Admittedly, these examples show the secretion of PACE using a recombinant DNA sequence encoding a full-length PACE. However, the secreted PACE is only a minor fraction of all recombinant PACE produced, resulting from a non-intended overproduction of full-length PACE by certain specific host cells, and only identified after a long incubation time, when the precursor polypeptide has already been completely processed to its mature form. Thus, the use of a recombinant DNA sequence encoding full-length PACE for obtaining secreted PACE cannot be seen - as argued by the respondent - as the only teaching derivable from these examples. On the contrary, by showing the possible shortcomings and drawbacks associated to such use of full-length PACE for obtaining secreted PACE - minor portion, long incubation, etc. - these examples further support the whole teaching or content of the application as filed, namely to use a recombinant DNA encoding a PACE lacking the transmembrane domain when a secreted PACE product is actually desired, such as it is the case for claim 7 as filed (cf point 7 *supra*).
9. Moreover, the factual situation in the present case cannot be compared to a situation wherein a list of equivalent products to be used in different possible methods is disclosed and the selection of a particular product with one of the many possible methods results in a specific combination, which has neither been disclosed in the application as filed nor contemplated by the skilled person on reading said application. In the present case, the use of both full-length PACE and PACE lacking the transmembrane domain in all disclosed methods, particularly in the co-expression method, represents distinct embodiments, which - even if not

specifically exemplified in the application as filed - are certainly contemplated in the whole content of said application. Both products are clearly identified as having different properties - located or anchored in the host cell membrane vs. secreted from the host cell - and thus, the reader is taught to select them as appropriate in connection with any other method step disclosed. Therefore, the established case law of the Boards of Appeal referred to by the respondent (cf point X *supra*) is not considered to be relevant to the present case.

10. The board concludes that the application as filed directly and unambiguously discloses a PACE lacking the transmembrane domain in the context of co-expression with a precursor polypeptide. Thus, the requirements of Article 123(2) EPC are considered to be fulfilled.

*Reimbursement of fees*

11. It remains to be assessed whether the patentees were denied a proper right to be heard (Article 113(1) EPC). In this respect and according to the "Minutes of the oral proceedings before the opposition division", the patentees were given time - at least twice - to consider the objection raised under Article 123(2)EPC. Afterwards, the patentees requested an adjournment of the oral proceedings in order to have an expert opinion from an independent expert (cf points 4.7 and 4.8 of the "Minutes of the oral proceedings before the opposition division"), which is also indicated in the decision under appeal (cf. point 7.1 of the decision under appeal). The minutes were not contested and thus,

they are assumed to correctly reflect the oral proceedings before the opposition division.

12. An objection under Article 123(2) EPC only requires assessment of the content of the application as originally filed, which the patentees and their representative are supposed to be well familiar with and to know in every detail. Only if there were a dispute as to what a particular term used meant to a skilled person in the art might expert evidence be needed, but this situation does not arise here. The assessment must be made by the instance of the EPO deciding the question. Given that the disclosure must be direct and unambiguous an opinion by an expert is likely to be of negligible assistance. In the present case, the patentees' main line of argumentation put forward before the opposition division as well as pages, passages, etc. of the application as filed given as a basis for the claimed subject-matter during the oral proceedings before the opposition division do not essentially differ from the ones indicated in the present appeal proceedings. The fact that the opposition division came to a different conclusion than the present board cannot be seen as a substantial procedural violation of the rights given by Article 113(1) EPC.

13. Moreover, the objection under Article 123(2) EPC had already been raised for the first time in the written submissions and not during the oral proceedings before the opposition division (cf page 4, lines 18 to 21 of opponent's letter dated 19 November 1999). Admittedly, it was raised among several other objections concerning the introduction into the claims of the word

"functional" and it might have been more clearly pleaded or more emphatically followed-up by the opponent. However, the objection was already formally present in the opposition proceedings and the patentees were on notice that they might have to deal with it.

14. In the light of these considerations, there was no need for an adjournment of the oral proceedings before the opposition division - with the associated delays, additional inconveniencies and problems - for allowing the consultation of an independent expert. The board does not consider that the right to be heard was denied in the present case and thus, no procedural violation against Article 113(1) EPC can be said to have taken place.

15. In the absence of a substantial procedural violation, the request for reimbursement of the appeal fees cannot be granted (Rule 67 EPC).

**Order**

**For these reasons it is decided that:**

1. The decision under appeal is set aside.
2. The case is remitted to the first instance for further prosecution on the basis of the sets of claims submitted at the oral proceedings on 6 November 2003.
3. The request for reimbursement of the appeal fee is refused.

The Registrar:

The Chairman:

A. Wolinski

L. Galligani