Datasheet for the decision of 14 October 2008

Case Number: T 0536/07 - 3.3.08
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, LLC, and Novartis Vaccines and Diagnostics, Inc.

Opponent:
Baxter Aktiengesellschaft

Headword:
Co-expression soluble PACE/GENETICS INSTITUTE

Relevant legal provisions:
EPC Art. 56, 54

Keyword:
"Main request - novelty (yes); inventive step (yes)"

Decisions cited:
T 1100/01, T 1329/04

Catchword:
-
Case Number: T 0536/07 - 3.3.08

DECISION
of the Technical Board of Appeal 3.3.08
of 14 October 2008

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(Patent Proprietors)
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Composition of the Board:
Chairman: L. Galligani
Members: P. Julià
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Summary of Facts and Submissions

I. European patent No. 0 574 402, based on the International application PCT/US91/08725 and published under the PCT as WO 92/09698 with the title "Expression of PACE in host cells and methods of use thereof", was granted with a set of claims for the Contracting States AT, BE, CH, DE, DK, FR, GB, IT, LU, NL, SE, and a different set of claims for the Contracting States ES and GR, both sets consisting of 8 claims.

II. The patent was opposed on the grounds as set forth in Article 100(a) EPC for lack of novelty and of inventive step. The opposition division considered that the amended claim requests filed during the opposition proceedings did not fulfil the requirements of Article 123(2) EPC and revoked the patent. The patentees appealed this decision and, in first appeal proceedings, the then competent board remitted the case to the opposition division for further prosecution on the basis of the set of claims submitted at the oral proceedings before the board (cf. T 1100/01 of 6 November 2003).

III. In the following opposition proceedings, the main request filed on 29 September 2006 was considered by the opposition division not to involve an inventive step and the patent was revoked.

IV. The patentees (appellants) filed a notice of appeal, paid the appeal fee and, under cover of a letter dated 11 June 2007, submitted a statement setting out the grounds of appeal with a main request and eight auxiliary requests. All requests comprised a set of
claims for the Contracting States AT, BE, CH, DE, DK, FR, GB, IT, LU, NL, SE, and another set of claims for ES and GR. Auxiliary requests 1 to 6 had already been filed before the opposition division and then withdrawn.

V. In a letter dated 22 October 2007, the opponent (respondent) replied to appellants' grounds of appeal.

VI. The board sent a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) informing the parties of its preliminary, non-binding opinion on substantive issues.

VII. In letters dated 11 and 12 September 2008, the respondent and the appellants replied, respectively, to the communication of the board. The appellants also filed auxiliary requests 9 to 12.

VIII. Oral proceedings took place on 14 October 2008. At the beginning of the oral proceedings, the appellants made the ninth auxiliary request filed on 12 September 2008 their new main request.

IX. Claim 1 of the appellants' main request for the Contracting States AT, BE, CH, DE, DK, FR, GB, IT, LU, NL, SE read as follows:

"1. A mammalian 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."

Independent claims 3 and 5 were directed, respectively, to a recombinant expression vector suitable for expression in a selected mammalian host cell and a method of increasing the yield of a biologically active protein comprising culturing a mammalian host cell, wherein in both claims the mammalian host cell was defined as in claim 1. Claims 2 and 4 were dependent on claims 1 and 3, respectively, and defined the precursor polypeptide. Claim 6 related to a specific embodiment of claim 5.

The main request comprised a corresponding set of claims for the Contracting States ES and GR. Claims 1 and 3 related, respectively, to methods of preparing a mammalian host cell and a recombinant expression vector suitable for expression in a selected mammalian host cell, wherein the mammalian host cell was defined as in claim 1 of the set of claims for the other Contracting States. Claims 2 and 4 were embodiments of claims 1 and 3, respectively. Claims 5 to 6 read as claims 5 to 6 of the set of claims for the other Contracting States.

In comparison to the main request underlying the decision under appeal and filed on 29 September 2006, the appellants' new main request required the host cell of independent claims 1, 3 and 5 to be a mammalian cell.
Moreover, claim 7 of that request which further defined the host cell of claim 5 (eukaryotic, insect, bacterial, yeast, etc.), was deleted in the new main request.

X. The following documents are referred to in the present decision:

D1: WO-A-91/06314 (publication date: 16 May 1991);


D20: EP-A-0 327 377 (publication date: 9 August 1989);


XI. The arguments of the appellants, insofar as relevant to the present decision, may be summarised as follows:

Main request
Novelty (Article 54(3),(4) EPC)

Document D1 was concerned with furin (in the contested patent designated as PACE), furin-like enzymes and
fragments or derivatives of furin or furin-like enzymes having endoproteolytic activity. Active furin fragments lacking the transmembrane domain were used in pharmaceutical compositions and in a process for in vitro cleavage of a precursor protein substrate. While these fragments were explicitly referred to in the context of extracellular cleavage, they were not mentioned in the context of (micro)biological processes for in vivo cleavage of precursor protein substrates. Although document D1 disclosed the co-expression of furin with a precursor protein substrate, there was no definition in this document suggesting that the term "furin" was short-hand for furin, furin-like enzymes and fragments thereof. This was confirmed when comparing claims 1 to 10 with claims 11 to 14. Whilst the former claims referred to active furin fragments as components of a pharmaceutical composition or as means for an in vitro method of cleaving a precursor protein substrate, claims 11 to 14 envisaged only the use of a furin or of a furin-like enzyme, but not a fragment thereof, in the (micro)biological production of a protein by culturing a host cell expressing a precursor form of this protein.

Inventive step (Article 56 EPC)

Documents D5 or D9 represented the closest prior art. Whereas document D5 disclosed mammalian host cells co-expressing PACE and pro-vWF (which resulted in a complete intracellular proteolytic processing of pro-vWF), document D9 disclosed the intracellular processing of pro-β-NGF by co-expressing either yeast KEX2 or human PACE. The contested patent disclosed co-expression systems which, in contrast to this
closest prior art, used a PACE lacking the transmembrane domain. The patent showed that minimal PACE fragments with enzymatic activity were capable of intracellularly processing (expressing and trimming) precursor protein substrates.

Starting from the closest prior art, the technical problem to be solved was the provision of an alternative co-expression system capable of processing a precursor protein substrate in an efficient manner. The solution was the claimed co-expression system in mammalian host cells which contained an expression vector that encoded a PACE lacking the transmembrane domain.

There was post-published evidence on file showing that the problem was solved. Documents D21 and D22 exemplified the claimed subject-matter with several precursor protein substrates (vWF, BMP2) and mammalian host cells (COS-1 and CHO). These documents showed that, contrary to the lower results obtained for endogenous pheromone substrates in yeast cells (cf. documents D4 and D20), a complete processing cleavage of the protein substrate was obtained in mammalian host cells. No knowledge other than that disclosed in the contested patent was required for obtaining the results of documents D21 and D22, i.e. these documents did not go beyond the teachings of the patent. Therefore, the present case was different from that underlying decision T 1329/04 of 28 June 2005, which was referred to by the respondent.

Although document D5 referred to a secreted, truncated PACE form, there was no characterization of this form.
Whereas the processing of pro-vWF by endogenous PACE was inefficient when pro-vWF was expressed alone (50% unprocessed and secreted into medium), co-expression with exogenous PACE resulted in a complete processing. Thus, document D5 aimed at improving the physiological machinery and intracellular processing within the cell. The importance of a proper localization in Golgi was known by the skilled person as shown by prior art on file (cf. documents D4 and D20). Similarly, document D9 aimed at an improved intracellular processing of a precursor protein substrate, which was efficiently obtained by co-expressing this protein substrate with PACE. The two PACE forms identified in document D9 were not characterized and there was no mention of a truncated, secreted PACE form. In view of the problem addressed by these documents and the efficient solution achieved by the disclosed co-expression systems, there was no motivation for the skilled person to look for further alternatives and certainly not to look for them in documents having a completely different goal.

In particular, document D20 was concerned with the production of secreted, soluble Kex2 forms for further purification and use in in vitro methods but not for using them in a physiological or in vivo situation as in documents D5 and D9. The deletion of the transmembrane domain prevented the retention of these Kex2 forms in the Golgi body and resulted in their secretion, allowing thereby an improved purification and use for cleaving precursor protein substrates under very determined conditions (in vitro medium). The advantages of these soluble Kex2 forms were associated only with their purification and isolation and the in vitro use of the purified forms. Document D20 disclosed
only artificial Kex2 forms constructed for a specific use and, in the absence of any suggestion to do so, there was no motivation for the skilled person to combine this document with the closest prior art. Although they could be combined, they would not be in the absence of such a suggestion.

The less so, since the prior art consistently taught that deletion of the Kex2 transmembrane domain caused mislocalization and improper or insufficient cleavage of precursor protein substrates (cf. documents D4 and D20). Soluble Kex2 forms were not properly located and they were not expected to be capable of participating in the intracellular maturation of precursor protein substrates since insufficient amounts were expected to be located in those compartments of the host cell in which precursor protein substrates were processed. The fact that in document D20 a low intracellular activity was shown with an endogenous substrate (killer factor) was only due to the nature of the assay (cell culture and pheromones), which allowed the detection of clear results with very small amounts of processed product.

XII. The arguments of the respondent, insofar as relevant to the present decision, may be summarised as follows:

Main request
Novelty (Article 54(3),(4) EPC)

Document D1 disclosed active furin fragments lacking the transmembrane domain for all uses, including the co-expression with a precursor substrate. Although in the passage of the description disclosing co-expression the term "fragment" was not explicitly mentioned, the
skilled person understood the term "furin or furin-like enzyme" used therein as including these active PACE fragments. There was nothing in document D1 to lead the skilled person to conclude that these fragments were unsuitable for co-expression. Nor that any of the furin fragments, derivatives or fusion proteins were in any way unsuitable or not contemplated for cleavage within cells.

The same standard had to be applied in assessing the teachings of document D1 and those of the contested patent. The patent disclosed the use of active PACE fragments lacking the transmembrane domain as isolated reagents or in the context of co-culture. However, it failed to mention these active PACE fragments in connection with co-expression. The definition found in the patent merely set out that the term "PACE" could include PACE fragments. Therefore, there was as much reason in document D1 to conclude that fragments were encompassed when the term "furin" was used as there was in the contested patent to conclude that all products disclosed in the patent were encompassed wherever the term "PACE" was used.

Inventive step (Article 56 EPC)

The closest prior art, represented by documents D5 or D9, disclosed the co-expression in mammalian cells of PACE and a precursor substrate. The technical problem to be solved was the provision of a mere alternative system. If the problem was more ambitious, namely the provision of an alternative system with an efficiency comparable to that of the closest prior art, neither the contested patent nor the post-published evidence on
file demonstrated that it had been solved. In fact, there was no example of co-expression with a soluble PACE in the contested patent and therefore, the claimed solution was not made plausible by the patent and, in line with decision T 1329/04 (supra), the supplementary post-published evidence could not be taken into account to establish whether or not the patent did indeed solve the purported problem.

In the present case, this supplementary post-published evidence did not even support appellants' arguments. Post-published document D21 did not provide any comparison yields between soluble PACE fragments and full-length PACE. Moreover, not all Kex2 substrates were shown to be PACE substrates, additional sequence requirements were necessary for PACE substrates and these requirements were not disclosed in the patent. Since the claims were not limited to any substrates (in the patent defined only as any pair of basic residues), a solution was not provided over the whole breadth of the claims. Similar deficiencies were identified in post-published document D22.

Whereas document D5 disclosed a secreted, truncated PACE form, document D9 referred to two PACE forms with different molecular weight and identified PACE as homologous to yeast Kex2. This information led the skilled person in a straightforward manner to documents D4 and D20, since they were concerned with the same technical problem, namely the processing and cleavage of precursor protein substrates by yeast Kex2. These documents showed that secreted Kex2 forms lacking the transmembrane domain retained the catalytic activity. Document D20 explicitly stated that the use of these
secreted Kex2 forms was advantageous and Table 2 showed them to have intracellular activity. Thus, active Kex2 fragments lacking the transmembrane domain were a feasible alternative to full-length Kex2 endoprotease. Since full-length Kex2 and PACE were homologous and functionally equivalent, there was nothing inventive in replacing the full-length PACE in the co-expression system of documents D5 or D9 by active PACE fragments lacking the transmembrane domain. This replacement represented only a non-inventive analogous substitution.

The distinction between intracellular or extracellular processing by PACE was not reflected in the claims. There was no reason for the skilled person to attach any importance to intracellular processing and indeed no importance was attached to it in the contested patent, which did not disclose whether the processing by PACE was intracellular, extracellular or a combination thereof. Although documents D5 and D9 indicated that endogenous proteins were cleaved intracellularly by Kex2 and PACE, there was no reason to consider it necessary in a non-physiological environment. In fact, document D20 clearly demonstrated Kex2 to be extracellularly active. Although the absence of the transmembrane domain had a negative effect on the biological activity of Kex2 in the context of endogenous substrate processing, there was nothing to suggest that this absence was also detrimental in processing an over-expressed exogenous precursor protein substrate, for which there was no "normal" localisation for processing and which was also known to be secreted from the host cell.
There was no reason to consider that intracellular processing was not to occur during co-expression with truncated PACE. Document D5 indicated that, when co-expressed with PACE, the processing of pro-vWF occurred at an earlier time than when expressed alone and processed by endogenous cleavage. Thus, PACE appeared to act on the precursor protein substrate whilst both were travelling through the secretory pathway. The skilled person expected the processing of pro-vWF whilst travelling through the secretory pathway regardless of whether or not the co-expressed PACE lacked the transmembrane domain. In fact, the use of truncated PACE was expected to combine the advantages of co-expression (convenience intracellular processing) with those of a shorter construct (less load on host cell) and the opportunity for further processing to take place in the culture medium. There was no prejudice against the replacement of full-length PACE by truncated PACE.

Document D5 indicated that, when pro-vWF was expressed alone in mammalian host cells, about 50% was secreted in a non-processed form into the medium. Similar results were described for Factor VII in the contested patent. Thus, the use of soluble PACE forms homologous to those active, soluble Kex2 forms of document D20 for treating the unprocessed pro-vWF in the extracellular medium was obvious.

XIII. The appellants (patentees) requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request filed as auxiliary request 9 on 12 September 2008.
XIV. The respondent (opponent) requested that the appeal be dismissed.

**Reasons for the Decision**

**Main request**

*Articles 123(2),(3) and 84 EPC*

1. No objections have been raised under these Articles. Nor does the board see any reason to raise any of its own motion.

*Novelty (Article 54(3),(4) EPC)*

2. Document D1, the sole document cited as anticipating the claimed subject-matter, concerns the human endoprotease furin which in the contested patent is designated as PACE (paired basic amino acid converting enzyme). Nevertheless, and contrary to the contested patent which defines the term "PACE" as including fragments that maintain the catalytic specificity of the enzyme (cf. page 5, lines 11 to 12 of the contested patent), document D1 consistently refers to "furin or a furin-like enzyme, or a fragment or derivative of furin or furin-like enzyme having an endoproteolytic activity". And each of these products is clearly defined in this document, in particular "furin-like" on page 4, lines 14 to 33, "furin fragment" on paragraph bridging pages 6 and 7, and "furin derivative" on page 7, lines 3 to 7. There is no indication that the term "furin" is to be understood as a generic term including all other products disclosed in the document.
3. When indicating that the proteolytic activity is maintained when the carboxy-terminal region with the transmembrane domain has been split off, document D1 states that "instead of the complete furin or furin-like enzyme, therefore, according to the invention, use can be made of a fragment of the enzyme which still contains the part responsible for the proteolytic activity" (cf. page 6, lines 30 to 35). However, there is no particular use mentioned in this context and, when the whole content of the document as mentioned above is taken into account, this reference cannot be interpreted as referring to each and every use disclosed in document D1.

4. This is even less so in view of the claims of document D1 which, in line with its description, are directed to embodiments comprising all disclosed products, namely claims 1 to 10 (pharmaceutical composition and a process for the in vitro cleavage of a protein), and to embodiments comprising only and exclusively "furin or a furin-like enzyme", namely claims 11 to 14 (a process for the (micro)biological production of a protein).

5. Thus, the requirements of Article 54(3),(4) EPC are considered to be fulfilled.

Inventive step (Article 56 EPC)

Closest prior art

6. Documents D5 and D9 are identified by both parties and in the decision under appeal as the closest prior art.

Document D5 is concerned with the "intracellular proteolytic processing of precursor polypeptides" and
the "maturation of many proteins including blood coagulation factors and growth factors". In order "to test propeptide cleaving activity of intracellular PACE", the document discloses the co-expression of a cDNA encoding the PACE and a polynucleotide encoding a precursor polypeptide substrate (pro-von Willebrand Factor, pro-vWF) for the encoded PACE in COS-1 cells. This co-expression results in the pro-vWF being completely processed. In contrast, when pro-vWF is transfected alone, the pro-vWF is correctly cleaved by an endogenous proteolytic activity but the process is inefficient since only about 50% of the secreted vWF is processed to the mature subunit. Document D5 further refers to the synthesis of a 90-100 kDa intracellular glycoprotein when PACE cDNA is expressed in COS-1 cells, "a portion of which was secreted into the medium as a truncated molecule".

Document D9 is concerned with the post-translational cleavage processing of inactive precursors of secreted bioactive proteins in mammalian cells. It discloses the co-expression of a cDNA encoding yeast KEX2 endoprotease or the human structural homologue furin with a polynucleotide encoding murine pro-ß-nerve growth factor (pro-ß-NGF) in BSC-40 cells. This co-expression "results in efficient conversion of the precursor to mature, biologically active NGF". Document D9 further states that, when cDNA furin is expressed alone in these mammalian cells, "two furin translation products (84 and 90 kD)" are revealed by immunoblot analysis.

7. The claimed subject-matter differs from the closest prior art in that a recombinant DNA sequence encoding a
PACE lacking a transmembrane domain replaces a DNA sequence encoding the full-length PACE in the co-expression systems according to the said prior art documents.

Technical problem to be solved and the proposed solution

8. Starting from the closest prior art, the objective technical problem to be solved is the provision of an alternative system to those disclosed in documents D5 and D9. The claimed subject-matter represents a solution to this problem.

9. Although there are no working examples for the claimed subject-matter in the contested patent and it is not disclosed as a preferred embodiment of the invention, there is a priori no reason for the skilled person to consider it not to be a plausible solution to the above mentioned technical problem. There is also post-published evidence on file demonstrating the feasibility of the proposed solution (cf. documents D21 and D22).

10. It has been alleged by the respondent that this evidence does not show the claimed subject-matter to be as effective as that of the prior art and that, therefore, it consists in a mere non-inventive worsening of this art. Moreover, in its view, in the light of the results disclosed in this later evidence, a solution is not provided over the whole breadth of the claims (cf. point XII supra). Having evaluated this post-published evidence, the board finds this argument unconvincing (cf. points 20 to 24 infra).
11. The present situation differs from that underlying decision T 1329/04 of 28 June 2005, wherein the then competent board decided that the claimed subject-matter did not provide a plausible solution to the identified technical problem. In that case, relevant structural differences between the claimed product and related products described in the art did not allow the former to be identified as a *bona fide* member of a family defined by the latter, even though such identification was later supported by post-published evidence that disclosed further characterizing features of the claimed product. In the present case, there is no indication whatsoever of a possible prejudice in the art or of foreseen difficulties in carrying out the proposed solution. Although the claimed subject-matter is not disclosed as a preferred embodiment in the contested patent, no further information is found in the post-published evidence that was not already made available to the skilled person by the contested patent (cf. points 20 to 24 *infra*).

**Obviousness of the proposed solution**

12. In line with the decision under appeal, the respondent considers the claimed subject-matter to be rendered obvious by the combination of either document D20 or D4 with the closest prior art.

Document D20 discloses several recombinant C-terminal truncated Kex2 yeast endoproteases, including variants lacking the cytoplasmic domain (pYE-KEX2Δ3, pYE-KEX2Δ4) and variants lacking the cytoplasmic, transmembrane and part of the extracellular domain (pYE-KEX2Δ1, pYE-KEX2Δ2, pYE-KEX2Δ6). Whereas the former variants retain all
Kex2 activity (killer and endoprotease activities), no activity is observed for the latter variants. Endoprotease activity is also retained for Kex2 variants lacking the cytoplasmic, transmembrane and a short part of the extracellular domain (pYE-KEX2(RI-PvuII), pYE-KEX2Δ5), even though a lower killer activity is reported (cf. Tables 2 and 3, pages 11 and 13). These variants, designated soluble Kex2 endoproteases, are extracellularly secreted and assumed not to bind to the yeast Golgi body (cf. page 3, lines 48 to 51).

Similar C-truncated Kex2 yeast endoproteases are disclosed in document D4. The most extensive deletion Δ6 corresponds to a Kex2 variant lacking the cytoplasmic, transmembrane and a short fragment of the extracellular domain (cf. Figure 2, page 483). Host cells transfected with a polynucleotide encoding the Δ6 deletion show an increase in extracellular Kex2 activity. Intracellular processing of the yeast pro-α factor is found only when the Δ6 deletion is produced at a high level (cf. page 484, left-hand column). It is assumed that the Δ6 deletion is unable to reside in the appropriate secretory compartment at a level sufficient to achieve the pro-α factor processing that takes place in the Golgi body, i.e. the Δ6 deletion fails to display a correct cellular localization (cf. page 483, left-hand column, last full paragraph and page 484).

13. In the light of these disclosures, the board considers that, in the absence of any specific pointers, hindsight is required to combine these documents with the closest prior art.
As stated above (cf. point 6 supra), both documents D5 and D9 relate to "intracellular proteolytic processing of precursor polypeptides" and disclose a particular co-expression system comprising several components (full-length PACE, precursor protein substrate) which are known to interact in a specific manner within a particular environment (mammalian secretory pathway, Golgi body). The board concurs with the respondent that the mere substitution of one of these components for another one having the very same properties, i.e. an analogous substitution, might \textit{prima facie} seem obvious to the skilled person as no significant alteration would be expected to occur in the interaction among all components within that particular environment. Indeed, document D9 already discloses such a substitution, since it reports similar efficient conversion of precursor protein substrate when co-expressing either human PACE or yeast Kex2 endoprotease. However, the substitution within this particular environment of one of the components for another one having different properties, i.e. a non-analogous substitution, is considered not to be straightforward in the absence of a hint or suggestion thereto in the art. It is the board's view that such a suggestion is not found in the present case.

14. Whilst document D5 discloses that the expression of PACE cDNA in COS-1 cells results in "a 90-100 kDa intracellular glyoprotein, a portion of which was secreted into the medium as a truncated molecule", there is no information on the quantitative importance of this portion nor on its possible presence and relevance, if any, in the co-expression studies. Although the skilled person might be interested in
assessing the possible relevance of this, such interest results more from idle scientific curiosity than from a specific technical purpose. Moreover, since there is no structural characterization of this truncated PACE form, let alone a functional one, it is not possible to equate it to any of the soluble Kex2 variants described in document D20. These Kex2 variants are not derived from an intracellular processing of a full-length Kex2 cDNA but from artificially cDNA constructs produced for a different purpose, namely to obtain active, soluble Kex2 endoprotease for isolation and further use as an extracellular reagent.

15. The importance of the intracellular processing in mammalian host cells cannot be dismissed offhand. In fact, both the unexpected presence of the secreted PACE form or the significant secretion of unprocessed precursor protein substrate when, as described in document D5, either the full-length PACE or the precursor protein substrate are expressed alone in COS-1 cells, are only unanticipated results of this intracellular processing (possible autocatalytic cleavage and saturated endogenous cleavage). To identify the mere reference to an uncharacterized, secreted PACE form in document D5 as a hint for the skilled person towards the active, soluble Kex2 variants of document D20 as well as a clear indication to replace the cDNA encoding the full-length PACE by a truncated cDNA encoding a soluble PACE form in the co-expression system of document D5 requires, in view of their different properties (targeted and retained in Golgi vs. mislocalized and not retained in Golgi) and the relevance of the intracellular processing, the benefit of hindsight.
16. There is no reference to any secreted PACE form in document D9, which refers to the detection of two translation products (84 and 90 kD) by immunoblot analysis when full-length PACE cDNA is expressed in BSC-40 cells. By immunofluorescence studies, these forms are localized in the Golgi. In document D9, PACE is identified as the human structural homologue of the Kex2 yeast endoprotease and the same co-expression results are described for both enzymes (cf. point 6 supra). As pointed out above, the attention of the skilled person might be drawn thereby to other Golgi-localized, membrane-associated endoproteases that may be used to obtain further analogous substitutions but not to the soluble Kex2 variants described in document D20, which are characterized by not being associated to a membrane and not binding to the Golgi body. Hindsight is thus required to combine this document with document D9.

17. It has also been argued by the respondent that, since it was known from document D5 that the production of recombinant precursor protein substrate alone saturates the endogenous proteolytic activity and results in the secretion of significant unprocessed precursor substrate, it would have been obvious to process this secreted precursor substrate using a secreted, soluble PACE form - equivalent to the soluble Kex2 yeast endoproteases of document D20 - by co-expressing cDNAs encoding both secreted products (cf. point XII supra).

18. Whereas it might be arguable whether the extracellular cleavage of unprocessed secreted precursor protein substrate using soluble PACE forms is an obvious
alternative to the disclosed co-expression of full-length PACE and precursor protein substrate, there is no hint in document D5 to lead the skilled person towards the co-expression suggested by the respondent. The suggested extracellular cleavage could be carried out by simply adding a soluble PACE form as a purified reagent (this being in fact the purpose of document D20 for obtaining soluble Kex2 variants) or by co-culturing host cells producing the precursor protein substrate and host cells producing soluble PACE forms. These alternatives actually correspond to the reagent and the co-culture embodiments of the contested patent. To read into document D5 a clear hint to the claimed subject-matter amounts to the application of hindsight.

19. It is also the board's view that the absence of a prejudice does not in itself constitute a clear motivation. It is only when this motivation is already given that the absence or presence of a prejudice might, respectively, encourage the skilled person to follow a particular lead or to disregard it as technical nonsense. As regards the references to the low killer activity of the soluble Kex2 variants described in document D20 or to the production of some mature α factor when the Δ6 deletion is produced at high level in document D4 (cf. page 11, Table 2, document D20; page 484, left-hand column, lines 4 to 11 from the bottom, document D4), they only appear to lead the skilled person away from the proposed solution and, in the present case, to be more relevant for assessing the expectation of success than for indicating any possible obviousness directly derivable from the closest prior art.
Post-published evidence and the scope of the claims

20. Document D21 discloses the same co-expression system as that referred to in document D5 (full-length PACE and pro-vWF in COS-1 host cells) and similar results are reported, i.e. secretion of 100% mature vWF (cf. page 2350, right-hand column, last full-paragraph and page 2351, Figure 1, lane 6). This document further refers to the co-expression of pro-vWF and a secreted, soluble PACE form lacking the transmembrane domain, which "efficiently processed vWF to its mature form" (cf. page 2352, paragraph bridging left- and right-hand columns). Although there is no quantitative comparison between both co-expression systems, similar results are shown when comparing their Western-blot analysis (cf. page 2351, Figure 1, lane 6 and page 2353, Figure 5B). Moreover, there is no reference to any particular condition or product (culture media, co-transfection, expression vectors, etc.) but only to standard ones (cf. page 2349, right-hand column).

21. Similar results are disclosed in document D22, which refers to the incomplete intracellular processing of recombinant human bone morphogenetic protein-2 (rhBMP-2) by the low amounts of endogenous PACE in CHO DUKX host cells (cf. page 32, middle column). Co-expression of rhBMP-2 with a soluble PACE form significantly reduces the presence of unprocessed and partially processed rhBMP-2 (cf. paragraph bridging pages 39 and 40). In this case, the cleavage of rhBMP-2 takes place earlier than - and in a different cellular compartment (endoplasmic reticulum, ER) from - that in which the endogenous PACE normally cleaves this precursor protein substrate (Golgi). There is no requirement for any
particular condition and the CHO DUKX host cells are known mutants of CHO cells lacking dihydrofolate reductase (DHFR) activity which are used for their easy selection with methotrexate (MTX), as shown in Example 5 of the contested patent. The unexpected advantageous effect of increased rhBMP-2 productivity cannot be taken into account for inventive step since it is not mentioned in the contested patent and it is not directly derivable therefrom (cf. paragraph bridging pages 40 and 41).

22. Based on the results of the substrate specificity studies of document D21, which identify the role of further basic amino acid residues at positions near the paired dibasic residues at the substrate cleavage site (lysine and arginine at positions two (P2) and four (P4) from the cleavage site), the respondent has argued that the contested patent does not provide a solution over the whole breadth of the claims since these sequence requirements were not disclosed in the patent (cf. point XII supra).

23. Although this objection relates more to Article 83 EPC and Article 100(b) EPC was not an original ground of opposition, the board acknowledges its possible relevance under Article 56 EPC as well. Nevertheless, the board cannot agree with the respondent since the contested patent identifies PACE as a "subtilisin-like endopeptidase, i.e. a propeptide-cleaving enzyme which exhibits specificity for cleavage at basic residues of a polypeptide, e.g. -Lys-Arg-, -Arg-Arg-, or -Lys-Lys-" and gives examples of possible precursor protein substrates for use in the disclosed methods (cf. inter alia page 5, lines 1 and 2, page 7, line 54 to page 8,
line 2). Whereas members of the subtilisin-like endopeptidase family might share a conserved functional cleavage site, such as the paired dibasic sequence disclosed in the patent, the substrate specificity profile (cleavage constant rate, binding affinity, etc.) was known to be specific for each member of this family and related to amino acid residues other than those of the conserved cleavage site (cf. inter alia page 484, Figure 3 of document D4). In vitro methods for determining preferred substrates of these enzymes were also available in the art (cf. inter alia, Example 3 of document D20).

24. If this objection is intended to show a lack of comprehensive knowledge of PACE at the filing date of the contested patent and the limitations of extrapolating any information based only on Kex2 yeast endoprotease, it must then be acknowledged that, at the filing date of the patent, even less information was available in the art regarding PACE and Kex2 variants lacking the transmembrane domain and, therefore, straight conclusions derived from information based only on these Kex2 variants (such as from documents D20 and D4) would be, to say the least, less than obvious.

**Conclusion**

25. It follows from all the above that the claimed subject-matter fulfils the requirements of Article 56 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 with the order to maintain the patent in an amended form on the basis of claims 1 to 6 of the main request filed as auxiliary request 9 on 12 September 2008 and a description and drawings to be adapted thereto.

The Registrar:     The Chairman:
A. Wolinski      L. Galligani