BESCHWERDEKAMMERN DES EUROPÄISCHEN PATENTAMTS

BOARDS OF APPEAL OF THE EUROPEAN PATENT OFFICE

CHAMBRES DE RECOURS DE L'OFFICE EUROPEEN DES BREVETS

Internal distribution code:
(A) [ ] Publication in OJ
(B) [X] To Chairmen and Members
(C) [ ] To Chairmen
(D) [ ] No distribution

D E C I S I O N
of 20 October 2004

Case Number: W 0010/04 - 3.3.8

Application Number: PCT/NL01/00936

Publication Number: -

IPC: C12N 15/90

Language of the proceedings: EN

Title of invention:
Nucleic said integration in eukaryotes

Applicant:
Universiteit Leiden

Opponent:
-

Headword:
Nucleic acid integration/UNIVERSITEIT LEIDEN

Relevant legal provisions:
PCT Art. 34(3)(a)
PCT R. 13.1-13.3, 68.2, 68.3(c), 68.3(e)

Keyword:
"Lack of unity (no)"

Decisions cited:
W 0006/90, G 0001/89

Catchword:
-

Europäisches Patentamt | European Patent Office | Office européen des brevets

Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: W 0010/04 - 3.3.8

International Application No. PCT/NL 01/00936

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 20 October 2004

Applicant: Universiteit Leiden
Stationsweg 46
NL-2312 AV Leiden (NL)

Representative: van Wezenbeek, L.A.C.M.
c/o Vereenigde
Nieuwe Parklaan 97
NL-2508 DH Den Haag (NL)

Decision under appeal: **Protest according to Rule 68.3(c) of the Patent Cooperation Treaty made by the applicants against the invention of the European Patent Office (International Preliminary Examining Authority) to restrict the claims or pay additional fees dated 15 October 2002.**

Composition of the Board:

Chairman: L. Galligani
Members: F. L. B. Brunel
M. B. Günzel

Summary of Facts and Submissions

I. International patent application PCT/NL 01/00936 (published as WO 02/052026) was filed on 21 December 2001 with 21 claims of which claims 1 to 4 read as follows:

"1. A method to direct integration of a nucleic acid of interest to a pre-determined site, whereby said nucleic acid has homology at or around the said pre-determined site, in a eukaryote with a preference for non-homologous recombination, comprising steering an integration pathway towards homologous recombination."

"2. A method to direct nucleic acid integration according to claim 1, comprising providing a mutant of a component involved in non-homologous recombination."

"3. A method to direct nucleic acid integration according to claim 1 or 2, comprising inhibiting a component involved in non-homologous recombination."

"4. A method according to claim 2 or 3, wherein said component involved in non-homologous recombination comprises *ku70*, *rad50*, *mre11*, *xrs2*, *lig4* or *sir4*."

Dependent claims 5 and 6 were directed to further features of the methods of claims 1 to 4. Independent claims 7 and 8 related to methods to direct integration of a nucleic acid of interest to a sub-telomeric and/or telomeric region in a eukaryote with a preference for non-homologous recombination. Dependent claim 9 was directed to further features of the methods of claims 7 and 8. Dependent claims 10 to 20 were directed to

further features of the methods according to anyone of the preceding claims.

Claim 21 was directed to the use of a method according to anyone of claims 1 to 20 for improvement of gene targeting efficiency.

II. On 15 October 2002, the EPO acting as an International Preliminary Examining Authority (IPEA) sent to the applicant an invitation to pay five additional examination fees pursuant to Article 34(3)(a) and Rule 68.2 PCT.

III. The invitation stated that there were six inventions claimed in the international application which were not linked by a single inventive concept.

The IPEA observed that the application aimed at improving homologous recombination (HR) by disabling non-homologous recombination (IR). The solution provided was the use of an eukaryotic cell with a preference for IR having either a mutated component involved in IR (claims 2 and 7), or the inhibition of a component involved in IR (claims 3 and 8).

The essential passages of the reasoning leading to the objection of lack of unity were as follows:

"T-DNA integrates in plant cells via IR. In S.cerevisiae and Aspergillus T-DNA carrying homology with the host genome integrates via homologous recombination (...), and T-DNA carrying no such homology integrates via IR (see **D16 and D18).** It is concluded that claim 1 is not novel over **D16** as well as

D18. D10 and D16 mention that the process of T-DNA integration is predominantly determined by host factors...**D6** discloses in particular (top of page 2) that "the control of HR or IR by modulating Rad50 provides the means to modulate the efficiency with which **heterologous nucleic acid** are incorporated into the genomes of a target plant cell. D6 refers to this modulation by regulation of the expression of Rad50 or by the inhibition of Rad50 (see page 41). D6 can therefore be considered to be prejudicial to the subject-matter of claims 1 and 3-4. In a similar way **D3** refers to a modulation by Ku70 (page 3 lines 10-14)."

The IPEA concluded: "Thus, the prior art has referred to the different effects of a number of host components (involved in IR) on recombination, as well as the preferred integration of foreign DNA at a pre-determined site by homologous recombination (see D18). It is therefore considered that at least the subject-matter of claims 1-3 is either not novel or inventive *[sic]* over the prior art documents D6 and D18. A single general inventive concept (...) is not recognisable anymore for the remaining subject-matter in the absence of a common, special technical feature."

IV. The following groups of separate inventions were listed:

i. the methods comprising as component ku70
ii. the methods comprising as component rad50
iii. the methods comprising as component mre11
iv. the methods comprising as component xrs2
v. the methods comprising as component lig4
vi. the methods comprising as component sir4.

V. On 14 November 2002, the applicant paid the additional fees under protest pursuant to Rule 68.3 PCT. The arguments submitted in favour of the protest insofar as they are relevant to the present decision were as follows:

– The applicant had identified for the first time certain proteins that played an essential role in the IR process of DNA integration and that did not play that role in the HR process of DNA integration. This finding formed the basis for the invention which provided a method by which the integration of DNA could be selectively "steered" towards HR.

– Neither **D6** (WO-A-00/68404) nor **D3** (WO-A-00/12716) taught that the inhibition of Rad50 (Ku70) resulted in selective integration of DNA via the HR mechanism. To the contrary, on the basis of either of these documents, the person skilled in the art would understand that inhibition of these factors would seriously negatively affect the integration capability of the target cell altogether.

D16 (Proc.Natl.Acad.Sci.USA, Vol.93, 1996, pages 15272 to 15275) and **D18** (Nature Biotechnology Vol.17, No.6, 1999, pages 598 to 601) respectively taught that DNA could be integrated in the *S.cerevisiae* or *S.awamori* genomes by the HR or IR processes depending on whether or not it contained sequences homologous to sequences in said genomes. Yet, none of them taught selectively steering DNA integration

towards HR in a eukaryote with a preference for IR.

For these reasons, it could be concluded that the novelty and inventive step objections raised by the IPEA were void and that claim 1 presented a single general inventive concept linking the various aspects of the invention.

VI. On 17 December 2002, the Review Panel of the IPEA confirmed the finding of lack of unity and invited the applicant to pay within one month a protest fee pursuant to Rule 68.3(e) PCT. The reasons given for justifying the invitation to pay additional fees were essentially as follows:

"D16 and D18 both steer an integration pathway towards HR by providing segments of DNA carrying homology to the target DNA. Moreover, D16 mentions that the integration is determined by the host organism, in particular host components involved in IR (in S.cerevisiae two genes RAD50 and TOP1).
D16 specifies that the T-DNA integration in S cerevisiae is at random positions via IR when the T-DNA lacks homology, and that segments of DNA varying homology with the S cervisiae *[sic]* genome integrate very efficiently into the yeast genome via HR. Moreover reference is made to host factors (like RAD50 and possible mutations thereof).

It is thus maintained that D16 destroys the novelty of the subject-matter of claim 1.

....: the method disclosed in D18 involves the replacement of normal IR by HR, at least partially (ie there is a steering of an integration pathway towards homologous recombination). The abstract of D18 refers to an efficient method for constructing recombinant mold strains.... Multiple copies of a gene can be integrated rapidly at a predetermined locus in the genome...

It is thus maintained that D18 is prejudicial to the novelty of claim 1".

Furthermore, the Review Panel stated that:

"In the final reasoning of the previous invitation (paragraph 4) concerning the lack of unity of invention it was mentioned that in particular **D6 and D18** makes the subject-matter of claims 1-3 obvious to the skilled person (thus a steering towards HR is possible): D6 making clear that Rad50 (one of the host components involved in IR) is a modulator of recombination (the modulating obtainable by inhibiting Rad50 gene expression; page 41), and D18 making clear that HR can be made efficient at pre-determined sites when DNA sharing homology is used. Therefore, the subject-matter of claims 1-3 does not fulfil the requirements of either Article 33(2) or (3) PCT.

It was therefore considered that the subject-matter of depending claim 4 relates to a group of different inventions which do not have a single general inventive concept (referred to in Rule 13 PCT and the PCT Preliminary Examination Guidelines Ch.III,7) in the

absence of a common special technical feature (novel and inventive)."

VII. On 17 January 2003, the applicant paid the protest fee and provided further arguments in reply to the decision of the Review Panel. The Applicant essentially maintained that neither D6 nor D18 affected the novelty of claim 1 as they failed to teach steering the pathway towards homologous recombination. Moreover, there was no incentive for the skilled person to combine the teachings of D6 (or D3) and D18 or D16.

Reasons for the decision

1. The protest in respect of the payment of five further examination fees is admissible.

2. According to the PCT regulations (cf. Rule 13.1 PCT), the international patent application shall relate to one invention only or to a group of inventions so linked as to form a single inventive concept. If the IPEA considers that the claims lack this unity, it is empowered, under Article 17(3)(a) PCT, to invite the applicant to pay additional fees.

3. Lack of unity may be directly evident *a priori*, ie before the examination of the merits of the claims in comparison with the state of the art revealed by the search (cf., for example, decision W 6/90, OJ EPO 1991, 436). Alternatively, having regard to decision G 1/89 of the Enlarged Board of Appeal (OJ EPO 1991, 155), the IPEA is also empowered to raise an objection *a posteriori*, ie after having taken the prior art

revealed by the search into closer consideration. This practice is laid down in the PCT International Search Guidelines Chapter VII-9. (PCT Gazette Special Issue, 8 October 1998, page 26)) which are the basis for a uniform practice of all International Searching Authorities. The Enlarged Board of Appeal indicated that such consideration represents only a provisional opinion on novelty and inventive step which is in no way binding upon the authority subsequently responsible for the substantive examination (point 8.1 of the Reasons for the decision).

4. In the present case, the IPEA raised a lack of unity objection *a posteriori* based on prior art documents D6, D3, D16 and D18 which were cited as being detrimental to the novelty or inventive step of the subject-matter of claim 1. As a consequence of this objection, the IPEA considered that six groups of separate inventions were claimed which were not linked together by a single inventive concept. Thus, the question to be answered is whether the unitary link represented by claim 1 is indeed affected by the said documents. Claim 1 relates to a method for integration of a nucleic acid of interest to a pre-determined site in a eukaryote with a preference for non-homologous recombination based on the concept of "steering an integration pathway towards homologous recombination". It, thus, must be assessed whether such a concept is known or obvious from the cited prior art.

5. D6 is a patent application describing the isolation of the maize Rad50 gene/protein as well as that of nucleic acids and proteins relating to maize Rad50 (Summary of the invention, pages 2 and 3). Definitions of the

technical terms used in the specification are given from page 3 to page 20. Said nucleic acids and proteins are described in detail from page 20 to page 30. All methods and tools which would potentially lead to gene isolation, protein expression and provision of recombinant hosts are described from page 30 to page 51. Modulation of the expression of the genes of the invention (ie *rad50* gene-like genes) by antisense or sense technology is mentioned on page 41, lines 6 to 21 and from page 51 to page 53. On pages 57 and 58, the possibility of isolating inhibitors of the Rad50-like polypeptides useful for the purpose of modulating the expression of said polypeptides is envisaged. From page 53 to page 57, a method of genotyping a plant comprising a Rad50-like polypeptide is described as well as further possible *rad50*-like polynucleotides. Finally, four examples are presented describing the isolation of the *rad50* cDNA/gene and giving the corresponding sequence of the Rad50 protein.

6. **At no point in the said specification** is a method disclosed which would take advantage of the envisaged in vivo modulation of the expression of the Rad50 protein for altering the natural balance between HR and IR. In fact, it is only in the chapter "Background of the invention" (pages 1 and 2) that the role of Rad50 and alike proteins (MRE11, XRS2) is mentioned, said role being their involvement in homologous (page 1, lines 20 to 24) **as well as** in illegitimate (page 1, lines 25 to 28) recombination. On page 2, it is stated: "Control of homologous recombination or non-homologous end joining by modulating Rad50 provides the means to modulate the efficiency with which heterologous nucleic acids are incorporated into the genomes of a target

plant cell." In the Board's judgment, this statement implies that **both** HR and IR could be **directly** affected by the modulation of the *rad50* gene expression. This **is not** a clear and unambiguous disclosure of a method which allows homologous recombination to occur in a preferential manner by steering the integration pathway.

7. For these reasons, it is concluded that D6 does not affect the novelty of the subject-matter of claim 1 and, thus, it does not anticipate the concept which is at the basis of the claim. The same is true of D3 which is the equivalent patent application in relation to the maize gene ku70.

8. D16 teaches that in higher organisms such as plants, IR is the predominant mechanism of DNA integration and that in *S.cerevisiae,* segments of DNA carrying homology with the genome integrate very efficiently via HR whereas IR events occur at a low frequency (page 15273, Introduction). Therefore, *S.cerevisiae* is not a eukaryote with a preference for IR. D16 is concerned with DNA integration in the *S.cerevisiae* genome, and thus, does not seem to be relevant for the assessment of the novelty of the claimed subject-matter.

9. D18 teaches that Ti DNA is transferred to the *A.awamori* fungal genome by IR but that HR may also occur when the Ti DNA carries DNA homologous to the *A.awamori* DNA (in the specific case, part of the *A.awamori pyrG* locus, page 600, first paragraph). Thus, D18 teaches a method for integration of a nucleic acid of interest (Ti DNA containing the *Fusarium solani pisi* cutanase gene linked to the part of the pyrG locus) to a pre-determined site (the pyrG locus on the genome) whereby

said nucleic acid has homology at or around the said predetermined site in a eukaryote with a preference for IR. However, the method involves the usual *A.tumefaciens*-mediated transformation. Otherwise stated, no specific steering step is taken so that integration via HR at the pre-determined site is favoured over integration via IR. The method, thus, does not rely on the concept of steering the integration towards HR. Accordingly, D18 is not detrimental to the novelty of claim 1.

10. Whether or not any of the disclosures of D6 (or D3) and D18 make in any way obvious the concept at the basis of claim 1 is the next point to be examined. As already mentioned in points 5 and 6, above, D6 teaches that Rad50 is involved in HR as well as IR and that either of these mechanisms can be controlled by modulation of Rad50 expression. That it might be possible to alter one pathway independently from the other is **nowhere suggested**. D18 shows that HR can occur in fungi, yet it is not concerned with improving the efficiency with which it will occur compared to IR. In the Board's judgment, neither of these documents are suited as starting point for defining the problem which the present invention purports to solve, which is that of favouring HR over IR. Nor is the concept on which the solution is based rendered in any way obvious. For these reasons, the inventive step of the subject-matter of claim 1 is not affected by either of these documents.

11. For these reasons, it is concluded on the basis of the analysis of the documents cited in the invitation to pay additional fees that the subject-matter of all claims is linked by a single inventive concept as

expressed in claim 1 and that, therefore, the requirement of unity of invention is fulfilled. The protest is justified.

Order

For these reasons it is decided that:

The refund of the five additional examination fees and of the protest fee is ordered.

The Registrar: The Chairman:

G. Rauh L. Galligani