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Datasheet for the decision of 24 September 2008

Case Number: T 0415/07 - 3.3.08

Application Number: 98924468.6

Publication Number: 0985032

IPC: C12N 15/10

Language of the proceedings: EN

Title of invention:
Ribosome complexes as selection particles for in vitro display and evolution of proteins

Patentee:
Discerna Limited

Opponent:
MEDIMMUNE LIMITED

Headword:
Ribosomal display/DISCERNA LTD.

Relevant legal provisions:
-

Relevant legal provisions (EPC 1973):
EPC Art. 83, 87, 88, 56

Keyword:
"Sufficiency of disclosure - yes"
"Priority entitlement - yes"
"Inventive step - yes"

Decisions cited:
T 0694/92, T 0792/00

Catchword:
-

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Case Number: T 0415/07 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 24 September 2008

Appellant: (Opponent)	MEDIMMUNE LIMITED Milstein Building Granta Park Cambridge Cambridgeshire CB21 6GH (GB)
Representative:	King, Hilary Mewburn Ellis LLP York House 23 Kingsway London WC2B 6HP (GB)
Respondent: (Patent Proprietor)	Discerna Limited Babraham Hall Babraham Cambridge CB2 4AT (GB)
Representative:	Davies, Jonathan Mark Reddie & Grose 16 Theobalds Road London WC1X 8PL (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted 19 December 2006 concerning maintenance of European patent No. 0985032 in amended form.**

Composition of the Board:

Chairman: L. Galligani
Members: F. Davison-Brunel
C. Rennie-Smith

Summary of Facts and Submissions

I. European patent No. 0 985 032 with the title: "Ribosome complexes as selection particles for in vitro display and evolution of proteins" was granted with 29 claims on the basis of the European application No. 98924468.6 corresponding to the international application No. PCT/GB98/01564 published as WO 98/054312.

II. An opposition was filed under Article 100(a) and (b) EPC for lack of novelty, inventive step and industrial application as well as for lack of a sufficient disclosure. The patent was maintained in amended form on the basis of a set of claims comprising claims 1 to 28 filed at oral proceedings.

Claim 1 read as follows:

"1. A method for the display and selection of proteins or peptides and for the recovery of the genetic material encoding them, which method consists of :

(a) transcription and translation of DNA in a eukaryotic cell free transcription/translation system such that complexed particles are formed, each comprising at least one individual nascent protein or peptide or other DNA expression product associated with one or more ribosomes and the specific mRNA encoding the protein or peptide;

(b) contacting the said complexed particles with a ligand, antigen, antibody or other agent in order to select the particles through binding to the protein or peptide product, and

(c) recovering the genetic information encoding the protein or peptide as DNA by means of reverse transcription and polymerase chain reaction (RT-PCR) carried out on the mRNA while the latter remains bound to the said complexed particle."

Dependent claims 2 to 5 related to further features of claim 1. Independent claim 6 was directed to a method in which DNA was produced by RT-PCR on an mRNA physically linked to eukaryotic ribosomes. Independent claim 7 was directed to a method having the same features as the process of claim 1 wherein step (a) was carried out in a cell free rabbit reticulocyte system. Dependent claims 8 to 21 related to further features of the previously claimed methods. Independent claims 22 and 23 respectively related to a method for making antibodies of a mouse, rat or other non-human mammals and a method for making human antibodies. Independent claims 24 and 25 respectively related to processes with the same features as the process of claim 1 whereby step (a) was said to be carried out by translating mRNA or an mRNA library (claim 24) or by transcribing and translating cDNA or a cDNA library (claim 25) in a eukaryotic cell free system. Dependent claims 26 to 28 related to further features of some of the previously claimed methods.

III. The appellant (opponent) filed an appeal, paid the appeal fee and submitted a statement of grounds of appeal.

IV. The respondent (patentee)'s reply to the grounds of appeal was followed by a further submission by the appellant.

V. The board sent a communication pursuant to Article 15(2) of the Rules of Procedure of the Boards of Appeal, indicating its preliminary non-binding opinion.

VI. The respondent's answer to this communication was accompanied by 13 auxiliary requests.

VII. In a letter dated 10 September 2008, the appellant's representative answered some of the points made by the respondent and informed the board that she would not be attending the oral proceedings.

VIII. At oral proceedings, the respondent replaced all claim requests on file with a sole main request which comprised 27 claims and corresponded to the claim request accepted by the opposition division except that claim 23 was deleted (see section II, supra).

IX. The documents mentioned in the present decision are the following:

(1): Mingyue He and Michael J. Taussig, Nucleic Acids Research, Vol.25, No.24, pages 5132 to 5134, December 1997;

(3): Hanes, J. et al., FEBS Letters, Vol. 450, pages 105 to 110, 1999;

(8): Matteheakis, L.C. et al., Proc.Natl.Acad.Sci. USA, Vol.91, pages 9022 to 9026, September 1994;

(9): Jozef Hanes and Andreas Plückthun, Proc.Natl. Acad.Sci.USA, Vol.94, pages 4937 to 4942, May 1997;

(12): Mingyue He and Michael J. Taussig, J. of Immunological Methods, Vol.297, pages 73 to 82, 2005;

(13): Gersuk, G.M. et al., Biochemical and Biophysical Research Communications, Vol.232, pages 578 to 582, March 1997;

(16): Xiang-Hua Yan and Zi-Rong Xu, Indian Journal of Biochemistry and Biophysics, Vol. 42, pages 350 to 357, December 2005;

(17): Rothe, A. et al., Journal of Biotechnology, Vol. 130, pages 448 to 454, 2007;

(18): Yong-Min Yang et al., Biophysical and Biochemical Research Communications, Vol.359, pages 251 to 257, 2007;

(19): Douthwaite J.A. et al., Protein Engineering, Design and Selection, Vol. 19, pages 85 to 90, 20 December 2005;

Declarations of Dr. J. A. Douthwaite dated 23 June 2004, 24 October 2005 and 30 April 2007;

Declaration of Drs M. He and M. J. Taussig dated 11 August 2008.

X. The appellant's submissions in writing insofar as they are relevant to the present decision may be summarised as follows:

Article 83 EPC; sufficiency of disclosure

- The patent did not provide a sufficient disclosure as regards, in particular, the method of claim 1, step (c) whereby the mRNA was said to remain bound to the ribosomal complex while RT-PCR was carried out. If the method steps were performed as taught in the patent, the mRNA would not remain bound to the complexed particle. The respondent's allegation, that the absence of reverse transcription observed when using a 3' end primer instead of an internal one constituted evidence that the mRNA remained bound to the complex, was not correct. It could simply be due to the fact that the 3' end of the mRNA was degraded by ribonucleases.
The appellant had filed data (declarations of Dr. Douthwaite of 23 June 2004 and 30 April 2007) showing that complexes were efficiently disrupted by the conditions used for reverse transcription, in particular the temperature, and furthermore that, contrary to the respondent's allegations, the mRNA could be reverse transcribed using a 3' end primer. The experimental differences in the method then used and the method in the examples of the patent in suit were not of the kind to affect RNA priming. In particular, the objection that the 3' end primer was too long, ie. that it was, in fact, operating as an internal primer, was unjustified as this primer adopted a secondary structure which made it no longer than the region supposed to be "protected" by the ribosome.

- Lack of sufficient disclosure also stemmed from the fact that in accordance with the description, the claimed method could only be performed if the reverse transcription was initiated with internal primers whereas the claims encompassed the use of 3' primers. The post-published documents filed by the respondent were not relevant to show that the claimed method would work over the scope of the claims because they all disclosed the method being carried out with internal primers.

- In accordance with such case law as T 694/92 (OJ EPO 1997, 408), there was insufficient disclosure if the skilled person could not carry out the invention over the full scope of the claims without undue burden and without relying on inventive skills. Here, it would require inventive skill to apply successfully the method of the patent using an RT-PCT primer directed to the 3' end of the mRNA since the patent taught away from doing so. As for T 792/00 of 2 July 2002, it established that the patent specification must put the skilled person in possession of at least one way of putting the claimed invention into practice over the whole scope of the claim. In the present case, he/she would need to carry out further investigations in order to confirm that it could be carried out by a technique that was different from what the description taught was required, namely an upstream primer with failure if it was not used.

For these reasons, the requirement of sufficiency of disclosure was not fulfilled.

Articles 87 and 88 EPC; priority entitlement

- Lack of priority arose from fact that the teaching in the priority applications was not enabling. Indeed, contrary to the patent in suit, it was not taught in these documents that magnesium ions must be present in the buffers used to wash the complexes during the step of selecting those which remained bound to the antigen-coated beads. In the absence of magnesium ions, the complexes would fall apart, the mRNA would be lost and the yield of DNA recovered by RT-PCR would be too poor to make the method usable in practice. There was experimental evidence to this point in the declaration of Dr. Douthwaite of 24 October 2005. The same information was found in eg. documents (3) and (12).

- Furthermore, in the priority applications, the claimed method for the display and selection of proteins was said not to require purification of mRNA at the start of a cycle nor the release of mRNA from the ribosome before RT-PCR at the end of a cycle. This information was not the same as stating that the mRNA remained bound to the complexed particle while RT-PCR was carried out, a feature found in present claim 1.

For these reasons, the claimed methods involving ribosomal display were not entitled to priority as from the filing date of any of the priority applications.

Article 56 EPC; inventive step

- Even if priority was acknowledged, the subject-matter of claim 1 lacked inventive step. Document (13) was the closest prior art as it disclosed a method of ribosome

display where the mRNA was separated from the ribosomal complex before RT-PCR. The appellant had provided comparative experimental evidence of this earlier method and that of the alleged invention. It had shown that there was no improvement in yield at all when carrying out the method as claimed. In the same manner, the post-published document (3) showed that the claimed method was less efficient than that of using a prokaryotic ribosome display system. It was obvious to save time by omitting a step that improved performance and the obvious result was a faster but less effective method. The claimed alternative method of ribosomal display thus lacked inventive step.

XI. The respondent's submissions in writing and during oral proceedings insofar as they are relevant to the present decision may be summarised as follows:

Article 83 EPC; sufficiency of disclosure

- The claimed method had been successfully chosen by different research groups for achieving their own purposes (cf. post-published documents (16), (17) and (18)).

As regards the feature in claim 1 that the RT-PCR reaction was carried out on the mRNA while the latter remained bound to the complexed particle (step (c)), it was consistent with the respondent's data showing that cDNA could not be generated by a primer which bound at the 3' end, which data had been published after scrutiny by peer review (cf. post-published document (12)). The allegation that the complexes would necessarily be disrupted at RT temperatures had not been substantiated

since the experiments which were performed by the appellant to show release of the mRNA had not been carried out under the RT conditions exemplified in the patent.
Moreover, the inventors had been able to show in experiments in which the ribosome complexes were intentionally disrupted that the mRNA 3' end had remained intact, which took care of the appellant's further argument that the absence of reverse transcription with a 3' end primer would be due to mRNA degradation.
The appellant's results which led them to conclude that the mRNA was detached from the ribosomal complexes - ie. the fact that they obtained reverse transcription with a 3' primer - could be explained in particular by the fact that their primer may have been long enough to act as an internal primer.

- Step (c) of eg. claim 1 had been so formulated as to leave no doubts as to the gist of the invention namely that, contrary to what was taught by numerous documents of the prior art (eg. documents (8) or (9)), there was no need to separate the mRNA from the ribosomal complexes before RT-PCR. The description provided a clear teaching as to which experimental conditions to use or not use in order to perform RT-PCR in situ. The skilled person was given all necessary guidance to be able to reproduce the invention.
There could have been a lack of sufficient disclosure in relation to a claim to a method specifically directed to carrying out RT-PCR with a 3' end primer but such a subject-matter was not claimed.

Articles 87 and 88 EPC; priority entitlement

The priority applications described the washing of the eukaryotic ribosomal complexes in buffers which did not contain magnesium ions. This feature did not prevent a successful outcome of the RT-PCR. Indeed, short washing steps in water or other magnesium-free buffers did not have the same disruptive effects on the ribosomal complex as a longer incubation at an elevated temperature as used in document (13) to disrupt wheat-derived ribosomal complexes. Improvements of the method as described in the priority applications could be sought for and one of them might be the addition of magnesium ions in the washing buffers but this did not mean that these ions were essential in such brief washes as were included in the claimed method. Both the inventors' publications (cf. post-published documents (1) and (12)) and also those of other scientists (cf. post-published documents (16) and (17)) all made use of magnesium-free washes while successfully carrying out the claimed invention.
Thus, the protocol given in the earliest priority document was enabling and the claimed invention was entitled to the earliest priority date of 28 May 1997.

Article 56 EPC; inventive step

Until the present invention, ribosome display and RT-PCR had consistently been regarded as two separate steps in the method for display and selection of proteins or peptides and for recovery of the genetic material encoding them. Indeed, the method of the prior art included a first step whereby the ribosomal complex comprising mRNA was panned by affinity selection to the

ligand of interest, then the mRNA was dissociated from the ribosomal complex before the second step of RT-PCR took place. This approach was considered as perfectly satisfactory. It was fully appreciated that the entities in play (ribosomes, mRNA, complexes etc...) were rare or quite sensitive to experimental conditions so that the skilled person - being naturally cautious - may have considered optimizing the method as it was already known to work, but would never have departed from it. Evidence thereto could be found in the fact that none of the documents of the prior art even hinted at the possibility of doing so. The inventors thus exercised inventive skills when setting up the now claimed method which required that both steps be physically linked, the RT-PCR being directly performed on the complex resulting from panning the ribosomal display.

XII. The appellant requested in writing that the decision under appeal be set aside and that the patent be revoked.

The respondent requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request filed during the oral proceedings.

Reasons for the decision

Main request filed at oral proceedings (claims 1 to 27: claims 1 to 22, 24 to 28 accepted by the opposition division)

Article 83 EPC; sufficiency of disclosure

1. The patent in suit discloses a method for identifying proteins with high affinity for a specific ligand and retrieving the corresponding encoding sequences. The method as illustrated with antibodies requires that a library encoding antibody fragments is transcribed and translated in vitro under such conditions that ribosomal complexes are formed which comprise the encoding mRNA - as a result of transcription -, the ribosomes and the antibody chains - as a result of translation. These complexes are reacted with the antigen of interest which is fixed on beads and, after washing, the complexes are submitted to a reverse transcriptase-polymerase chain reaction (RT-PCR) whereby the mRNA is read into cDNA which is then amplified.

2. The unambiguous teaching of the description is that performing RT-PCR on the complexes amounts to using as a template an mRNA the 3' end of which is embedded in the ribosomes, which, in turn, brings in the limitation to the reverse transcription reaction that it must be carried out with an internal primer - the 3' end primer not being able to hybridize to the hidden mRNA 3' end. This point is repeatedly emphasized throughout the description eg. on page 16, paragraph 5 of the application as filed or in Example 1. In fact, this example is specifically intended to demonstrate that the 3' end of the mRNA is not accessible to the 3' end

primer whereas an internal primer is effective. Examples 10 and 12 describe the selection of an antibody fragment with high affinity to progesterone or testosterone from mixture of mutated antibody fragments or from a library of antibody fragments. There is no doubt on the basis of the teaching in the patent in suit and of the confirmation in post-published documents (cf. documents (16) to (18)) that the method can be put into practice.

3. The dispute is rather whether the molecular mechanism which results in the cDNA reverse transcript - identified in claim 1, step (c) as "by means of reverse transcription and polymerase chain reaction (RT-PCR) **carried out on the mRNA while the latter remains bound to the said complexed particle**" (emphasis added)- is, indeed, the correct one. Numerous detailed experiments and counter-experiments or references to scientific publications have been filed/cited by the parties in support of/ against the existence of this mechanism. In fact, this divergence of views seems to have been going on for more than five years (see document(12), paragraphs 2.7 and 3.3 and page 80, right-hand column and document (19), page 88, passage bridging the left- and right-hand columns, both published in 2005, which report opposite results).

4. At this point, it should be understood that it is not within the board's capacities nor indeed its duties to settle a scientific dispute such as this. The question to be answered is simply whether or not the method can be carried out when, as taught by the patent in suit, the selected antigen-coated beads-ribosomes-nascent antibodies- mRNA complexes are put directly in the presence of reverse transcriptase under conditions of

reverse transcription. And, as already above mentioned, there is evidence in the patent itself and also in post-published documents on file that this question can be answered positively. Thus, it is concluded that the reproducibility of the claimed method is not affected by the fact that there is no agreement as to the molecular mechanism involved.

5. The appellant also pointed out that, whereas claim 1 relates to a generic teaching which included the possibility of performing reverse transcription with a 3' primer, in the description the reverse transcription was taught to be achievable only with an internal primer and, therefore, argued that sufficiency of disclosure was not achieved over the scope of the claim.

6. One can probably always think of an embodiment of a generic claim which may not work. Yet, what is somewhat unusual in this specific case is that the description itself brings this embodiment to attention. Of course, this also means that the description leaves no doubt as to how reverse transcription should be carried out - with an internal primer - and as to how it cannot be carried out - with a 3' primer. In other words, it teaches the conditions in which the method will work and warns the skilled reader as to what not to do. Under such circumstances, it becomes difficult to conclude that performing the invention requires undue burden or the exercise of inventive skills. In fact, in the board's judgment, the appellant's argument amounts to objecting that an essential feature is missing from the claim, namely that the reverse transcription must be carried out with an internal primer. This is an objection under Article 84 EPC. Yet, claim 1 is

identical to granted claim 1 and lack of clarity is not a ground of opposition. Accordingly, the argument must fail.

7. In its written submissions, the appellant mentioned two earlier decisions of the boards of appeal (T 694/92 and T 792/00, supra) as relevant to the present case. T 694/92 establishes that, for the requirements of Article 83 EPC to be satisfied, the skilled person should be able to carry out the invention over the scope of the claims. While the board fully agrees with this conclusion, it does not consider it as straightforwardly applicable to the circumstances of this case, for the reasons mentioned in point 6. As for T 792/00, it established that the patent specification must put the skilled person in possession of at least one way to perform the invention and this is clearly the case here (use of an internal primer), so it is not considered relevant case law.

8. For the above mentioned reasons, it is concluded that the requirements of Article 83 EPC are satisfied.

Articles 87 and 88 EPC; priority entitlement

9. The main question to be answered is whether the priority applications provide an enabling teaching of the claimed method although they do not mention the presence of magnesium ions in the buffers used to wash the complexes before RT-PCR. Indeed, in the absence of magnesium ions, the mRNA could be released from the complex in the washing buffers and, therefore, be lost for the RT-PCR. In this respect, it is observed that the patent

application itself discloses washing buffers containing magnesium ions.

10. In this respect also as with the necessity or not for an internal primer for reverse transcription, numerous experimental data and documents are cited by each party to argue in favour/against the presence of magnesium ions in the washing buffers being an essential feature for carrying out the invention. In the post-published document (19) (page 89, right-hand column), the presence of magnesium is recommended as a means to stabilize the ribosomal complex. In the prior art document (13), (page 579, left-hand column), it is taught that an elution step carried out for 15 minutes at 37°C in the absence of magnesium ions leads to the disruption of ribosomes. The post-published document (17), (page 449, par.2.5) describes the use of magnesium ions in washing buffers. In contrast, in the post-published documents (1) (page 5133, left-hand column) and (16), (page 353), the method is carried out with washing buffers not containing magnesium. Furthermore, it is remarked in document (19) that eukaryotic ribosomes are difficult to dissociate in the presence of EDTA which is a known magnesium chelator.

11. In their declaration of 11 August 2008 filed with the respondent's submissions of 22 August 2008, the inventors do not disagree with the fact that ribosome complexes may be disrupted in the absence of magnesium ions, yet they argue that it would very much depend on the experimental conditions. In particular, they express the opinion (cf. paragraph 26) that eukaryotic ribosome complexes withstand brief washings in water without magnesium. On this basis, the respondent argued that the

presence of magnesium ions in the washes should be regarded as an optimization of the claimed method rather than as an essential feature.

12. The board is prepared to give the respondent the benefit of the doubt, if only because document (1) published after scrutiny by peer review shows that the method may be carried out without magnesium ions in the washing buffers. The priority applications are, thus, considered as enabling as regards the claimed invention.

13. The further argument was presented that none of the priority applications provided an expressis verbis disclosure of the molecular mechanism discussed above (see point 3). This argument is not relevant to priority inasmuch as carrying out the claimed invention does not require scientific knowledge at that level.

14. The claimed subject-matter enjoys priority as of the filing date of the first priority application, namely 28 May 1997.

Article 54 EPC; novelty

15. There are no documents on file with a publication date preceding the 28th May 1997 disclosing a method identical to any of the methods now claimed. Novelty is thus acknowledged.

Article 56 EPC; inventive step

16. The closest prior art is document (13) which teaches a eukaryotic ribosome selection method to isolate peptide ligands which bind with high affinity with the Prostate-

Specific-Antigen (PSA). The ribosome libraries are characterized by the physical linkage of each peptide to its encoding mRNA in the ribosomal complexes. After selection of the complexes which bind to PSA-coated beads, these complexes are disrupted and the mRNA encoding the binding peptides is separated by elution under conditions leading to disruption of the complexes (page 579, left-hand column). The mRNA thus recovered is then reverse transcribed and amplified by PCR.

17. Starting from the closest prior art, the problem to be solved can be defined as providing an alternative method to the method described in document (13).

18. The solution provided is a method which differs from the method in document (13) in that reverse transcription is carried directly on the eukaryotic ribosomal complexes selected for their ability to bind to the protein coated particles. The claimed method is a bona fide alternative solution since there is evidence in the patent in suit and on file that it has been successfully performed (see point 2, supra).

19. The prior art shows that before the priority date, different methods of ribosome display followed by screening and recovery of the relevant genetic material had already been used (cf. documents (8) and (9)). These methods correspond to that described in document (13) except that the ribosomal display involves an E.coli in vitro transcription-translation system rather than a eukaryotic one. Quite irrespective of how ribosomal display is achieved, it remains that RT-PCR is always carried out on a mRNA which has been **deliberately** dissociated from the ribosomal complexes. In fact, none

of the prior art documents ever suggests that this part of the method could be modified. Moreover, it can be seen from eg. the post-published documents (3) or (19) that before the priority date and even thereafter, the procaryotic in vitro expression system continued to be thought satisfactory:

- Document (3), published in 1999, abstract: "In summary, we could not detect any intrinsic advantage of using a eukaryotic translation system for ribosome display."

- Document (19), published in 2006, page 89: "These results highlight that the method of mRNA recovery should be appropriate and optimal for the nature of the ribosomal display system and that direct transfer of a prokaryotic method to a eukaryotic system may result in a very inefficient process."

In other words, the skilled person was content with the methods available and had no incentive to depart from them. In addition, in view of the number and complexity of the molecular events necessary to get to the end result ie. to the DNA encoding the peptide/protein/antibody with the relevant binding properties, it is reasonable to expect that he/she might have been reluctant to do more than improve specific features of the methods as were known to work.

20. Under such circumstances, developing a method different from those of the prior art cannot have been obvious even in the absence of an explicit warning that it could not be done. Thus, in the board's judgement, it required inventive step to do so.

21. The argument was presented that when wanting to simplify a method, it would be obvious to omit a step and that a gain of time and loss in efficiency resulting there from would equally be expected. This argument is not relevant as it can only be made with the exercise of hindsight. As already mentioned, there is no evidence on file that a simplification of the existing methods would ever have been envisaged.

22. For the reasons given in point 19 supra, inventive step is acknowledged.

Adaptation of the description

23. Page 5 of the description has been amended in order to reflect the deletion of claim 23 which had been allowed by the opposition division (cf. section VIII, supra). There are no objections under the EPC to this amendment.

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 on the basis of the following documents:

- Pages 2 to 4 and 6 to 14 of the description as granted; page 5 of the description as filed during the oral proceedings;
- Claims 1 to 27 of the main request filed during the oral proceedings:
and
- The figures as granted.

The Registrar The Chairman

A. Wolinski L. Galligani