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D E C I S I O N
of 16 March 2005

Case Number: T 0239/01 - 3.3.4

Application Number: 92922343.6

Publication Number: 0566714

IPC: C12P 21/02

Language of the proceedings: EN

Title of invention:
Coupled transcription and translation in eukaryotic cell-free extract

Patentee:
Promega Corporation

Opponent:
Roche Diagnostics GmbH

Headword:
Coupled transcription and translation/PROMEGA

Relevant legal provisions:
EPC Art. 56

Keyword:
"Inventive step - (no)"

Decisions cited:
-

Catchword:
-

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Case Number: T 0239/01 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 16 March 2005

Appellant: (Opponent)
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Decision under appeal:
Interlocutory decision of the Opposition Division of the European Patent Office posted 3 January 2001 concerning maintenance of European patent No. 0566714 in amended form.

Composition of the Board:

Chair: U. M. Kinkeldey
Members: G. L. Alt
S. C. Perryman

Summary of Facts and Submissions

I. European patent No. 0 566 714 with the title "Coupled transcription and translation in eukaryotic cell-free extract" was granted on European patent application No. 92 922 343.

II. The patent was opposed by two opponents on the grounds as set forth in Article 100(a) EPC that the invention lacked novelty (Article 54 EPC) and inventive step (Article 56 EPC), Article 100(b) EPC and Article 100(c) EPC.

III. The opposition division maintained the patent on the basis of an amended main request.

IV. Opponent 01 lodged an appeal against this interlocutory decision.

V. The respondent (patentee) submitted observations with regard to the statement of the grounds of appeal. One further submission was received from each party in response to the communication accompanying the summons to oral proceedings. The respondent included an amended main request.

VI. Oral proceedings were held on 15 and 16 March 2005.

In the course of the oral proceedings a further amended main request was filed. The appellant did not raise objections under any of Articles 123(2)(3), 84, 83 and 54 with regard to this request.

Claim 1 of this main request read:

"1. A method of carrying out transcription and translation in a batch reaction from cloned or amplified DNA template comprising a specific RNA polymerase promoter in eukaryotic cell-free extract comprising adjusting the magnesium concentration of said extract to a level such that transcription and translation are coupled in the sense that RNA is transcribed from DNA and, simultaneously therewith, RNA translates into protein, wherein said extract is rabbit reticulocyte lysate and the final magnesium concentration is 2.5mM to 3.5mM or wherein said extract is wheat germ extract and the final magnesium concentration is 3.0mM to 5.25mM."

The request contained further dependent and independent claims.

VII. Reference to the following documents is made in this decision:

D1: EP-A-593 757

D15: Proceedings of the National Academy of Science, vol. 72, 1975, pages 1922 to 1926, Roberts et al.

D23: Nucleic acids Research, vol. 20, 1992, pages 4987 to 4995, Craig et al.

Declaration of Prof. Erdmann dated 9 October 1999

VIII. The submissions made in writing and during the oral proceedings with regard to the main request by the appellant, insofar as they are relevant to the present decision, may be summarised as follows:

Inventive step (Article 56 EPC)

Document D1 was the closest prior art document. It disclosed a continuous-flow method of preparing proteins in a cell-free system starting from DNA as a template.

The system disclosed in document D1 was **coupled** and not linked. Otherwise, the increase of the concentration of the produced protein per time unit during the whole process as shown in Figures 4 to 6 of document D1 would not be explainable.

Thus, since document D1 had already recognized the possibility of coupled transcription and translation, there could be no prejudice in this respect.

There were no doubts about the feasibility of a conversion of a continuous-flow method to a batch method because it was evident that the starting conditions of any continuous-flow method resembled those of a batch method. Additionally, once the skilled person had chosen the batch mode, it would not come to his mind to try to overcome the inherent disadvantages of this type of process vis-à-vis the continuous type. In contrast, he would accept the inherent disadvantages in view of the inherent advantages of the batch mode process, these being the reason of choosing it.

The provision of the claimed magnesium concentrations did not involve an inventive step, either. Firstly, the skilled person knew about the importance of the concentrations of this ion, for example from documents D1 or D15. Therefore, secondly, driven by its naturally present scientific curiosity, the skilled person would perform routine tests in order to determine the suitable range of magnesium ion concentrations for wheat germ extract and rabbit reticulocyte lysate.

IX. The submissions made in writing and during the oral proceedings with regard to the main request by the respondent insofar as they are relevant to the present decision, may be summarised as follows:

Inventive step (Article 56 EPC)

Document D1 was the closest prior art document. However, it disclosed a process in which transcription and translation were **linked** and not coupled. This conclusion could be drawn in view of the Erdmann-declaration describing a reproduction of the transcription/translation reaction of document D1. From the protocol it could be inferred that, when the ingredients were mixed, the translation lysate was the last ingredient added. Moreover, in view of the fact that the lysate was stored in a freezer at -20°C, it had even to be taken from the freezer and thawed before it was added. Therefore, transcription occurred first and was finished before translation could take place.

The prior art reflected a prejudice against the carrying-out of coupled reactions. This prejudice remained even after the filing of document D1 as evidenced by document D23.

There was nothing in the prior art confirming that a continuous reaction would work in a batch mode.

Finally, even if document D1 was deemed to disclose a coupled reaction, it did not hint at adjusting the magnesium ion concentration. In the light of document D1 the skilled person would rather have focussed on removing the disadvantages appearing when converting continuous to batch reactions. Moreover, the magnesium ion concentrations used in the process of document D1 were outside the claimed ranges.

X. Requests

The appellant (opponent) requested that the decision under appeal be set aside and that the European patent No. 0 566 714 be revoked.

The respondent (patentee) requested that the decision under appeal be set aside and that the patent be maintained on the basis of the claims of the Main Request submitted at the oral proceedings on 15 March 2005.

Reasons for the Decision

Articles 123(2)(3) EPC

1. The amendments to claim 1 have the following basis in the application documents as originally filed:

a) addition of the expression "in a batch reaction"

This amendment finds a basis in the disclosure on page 7, last paragraph continued on page 8, first paragraph and in all examples.

b) amendment of the phrase "...from DNA..." to "..from a cloned or amplified DNA template comprising a specific RNA polymerase promoter ..."

This amendment is based on page 12, lines 5-7.

c) inclusion of final magnesium concentrations for wheat germ extract and rabbit reticulocyte lysate definitions

These concentrations are found in claims 2 and 3 and claims 22 and 23 as originally filed.

Hence, the amendments comply with the requirements of Article 123(2) EPC. Since the amendments cited above are of limiting nature and were added to claim 1 as well as to the other independent claims, the Board is also satisfied that in conformity with Article 123(3) EPC the protection conferred by the claims is not extended.

Articles 54, 83 and 84 EPC

2. The appellant did not raise objections under any of Articles 54, 83 and 84 EPC with regard to claim 1 and the Board also does not see a reason to raise one.

Thus, the only remaining issue to be decided is that of inventive step.

Inventive step (Article 56 EPC)

The closest prior art and its teaching

3. Document D1 is regarded as the closest prior art document by the parties and the Board. There is disagreement, however, with respect to its disclosure content. Specifically, the question arose whether it disclosed a **coupled** or a **linked** transcription/ translation system. Thus, before the problem-solution approach can be correctly applied, the teaching of document D1 has to be determined.

4. Document D1 relates to a method of preparing polypeptides in a cell-free system starting from DNA as a template. "Cell-free" means that translation and transcription occur in the absence of whole cells. As far as the necessary enzymes are concerned, the document refers to T7 polymerase or SP6 polymerase as transcription enzymes and to E. coli, wheat germ embryo or rabbit reticulocyte lysates for translation. The lysates contain all components of the translation machinery, but are free of endogenous mRNA and DNA. Thus, the process is suited to produce heterologous proteins.

The process is run as a continuous-flow process, i.e. there is a continuous flow of reactants into and a continuous flow of products out of the reaction system. Thus, a continuous process is characterized by the use of continuous quantities of material. This can be contrasted with a batch process which is characterized by discrete, limited quantities of material.

5. The respondent is of the opinion that document D1 discloses a linked and not a coupled transcription/ translation system. The terms "linked" and "coupled" have the following meaning: It is known that the optimum ionic requirements of the transcription and translation reaction differ and that one of the necessary ions is magnesium. Generally, the transcription reaction needs higher magnesium ion concentrations than the translation reaction.

If one attempts to carry out both reactions in one vessel, one way of overcoming these disparities in, inter alia, the magnesium ion requirement, is to run the transcription reaction first, stop it by changing the conditions to those optimal for translation which then subsequently occurs. Such a system is called "linked". Thus, in a linked system two reaction steps take place in the same vessel, but separated in time. Document D15, for example, discloses such a linked system. The transcription reaction mixture in a buffer containing 10mM magnesium acetate is incubated for fifteen minutes. This is followed by a three hour incubation after the addition of the translation mixture which yields a final concentration of magnesium ion of 3mM (page 1923).

This linked system can be contrasted with a "coupled" system where transcription and translation occur in the same vessel at the same time without changing the ionic reaction conditions. This is possible because the selected ion concentrations are a compromise between the optima of the two reactions.

6. The respondent's argument that document D1 discloses a linked system is based on the following observations:

The Erdmann-declaration reports the reproduction of the D1-process and indicates on page 138 that the translation lysate is added at the very end, i.e. after addition of the nucleotide-mix, DNA-to-be-transcribed and RNA polymerase. On page 135 of the protocol it is stated that the translation lysate is stored at -20°C. Therefore, the respondent argues that the period for removing the lysate from the freezer, thawing and adding it to the reaction mixture would be so long that in the meanwhile transcription would have been finished. Thus, the experimental situation in the Erdmann-declaration and therefore also that in document D1 gives rise to a linked system where transcription and translation are separated in time and not to a coupled system where they occur at the same time.

7. The Board is not convinced by this line of argument.

7.1 In the absence of proof to the contrary it is accepted that the Erdmann-declaration discloses, as far as the order of mixing and storage of the lysate is concerned, an experimental set-up that is truly imitating that of document D1. Thus, for the following considerations a

reference to the Erdmann-declaration can be replaced by a reference to document D1.

7.2 The respondent submits that the delay leading to the completion of transcription before the start of translation is caused by taking the lysate from the freezer and thawing it before adding it to the mixture. The Board considers this scenario as highly improbable. The Board is of the opinion that a skilled laboratory worker would collect all necessary ingredients, thaw them, store them cooled on ice on the bench and mix them before he would put them at the correct temperature for the reaction to proceed. Thus, the long delay alleged by the respondent would not have occurred, with the consequence that transcription would not be completely finished before translation occurred. Thus, even when taking into account a short delay before adding the lysate, for example caused by changing pipette tips, the largest part of the reaction of document D1 must have been coupled.

8. Support for this view and thus the coupled nature of the process disclosed in document D1 comes from its Figures 4 to 6 relating to the amount of protein produced. RNA is a relatively unstable molecule. Thus, if, when following the respondent's argument, the complete amount of mRNA had been produced at the beginning of the reaction, this initial amount of RNA would immediately be reduced by degradation. Thus, even assuming that one mRNA molecule is translated more than once, the amount of protein produced per unit of time should become lower the longer the reaction runs due to the decrease in the number of mRNA molecules. Figures 4 to 6 of document D1 show however an increasing amount

of protein per time unit during the whole observed reaction period which is nearly 25 hours in Figure 4, approximately 33 hours in Figure 5 and approximately 20 hours in Figure 6. In the Board's view this picture can only be explained by assuming that RNA is continuously re-produced as in a coupled reaction.

9. Thus, the Board concludes that document D1 discloses a continuous-flow, coupled process for the cell-free production of proteins.

The problem to be solved

10. In view of this prior art and in the absence of evidence of any improvement achieved over the process disclosed in document D1, the problem to be solved by the patent in suit can only be formulated as the provision of an alternative method for carrying out transcription and translation from cloned or amplified DNA comprising a specific RNA polymerase promoter in eukaryotic cell-free extract which is either wheat germ extract or rabbit reticulocyte lysate and in which method the magnesium ion concentration is such that transcription and translation are coupled.

The solution

11. The solution to this problem provided by claim 1 of the patent in suit is a system running in a batch mode and in which the final magnesium concentration in the case of rabbit reticulocyte lysate is 2.5mM to 3.5mM and in the case of the wheat germ extract is 3.0mM to 5.25mM.

12. Protein production by this method is convincingly demonstrated in the examples of the patent in suit. Therefore, the problem can be regarded as solved.

Obviousness of the solution

13. The process of the patent in suit differs from the process of document D1 in two features, one relating to the general way of how the process is performed - continuous-flow versus batch - and the other to the reaction conditions for coupled transcription and translation - specific values of magnesium ion concentration versus a range of magnesium ion concentration. The claimed subject-matter could be regarded as inventive if at least one of the features would not be derivable from the prior art in an obvious way.

The feature "in a batch reaction"

14. Most of the documents that were submitted during these proceedings, for example document D15, describe batch reactions, therefore it can be concluded that this way of performing a reaction belongs to the common general knowledge. Consequently, to run the coupled, cell-free transcription/translation process described in document D1 in a batch mode would be an obvious choice for a skilled person seeking for an alternative process.

15. The respondent's argument that a person skilled in the art would not have chosen the batch method because he does not have a reasonable expectation of success that the continuous flow method of document D1 would also work in batch mode, does not convince the Board. In the

Board's view, the skilled person would have recognized the fact that at least in their initial phases, the conditions of a batch and a continuous-flow reaction are similar and that, therefore, for a restricted period of time, the reaction would take place in a batch mode, if the conditions of the continuous mode were chosen. Thus, the skilled person would have expected success.

16. The disadvantages of a batch reaction in contrast to a continuous reaction, such as for example the shorter running time and consequently the lower amount of produced protein due to the non-replaced consumption of reactants during the reaction, would also not have prevented the skilled person from choosing this type of reaction. Rather, he would have put up with them knowing about their inherent presence, but knowing also about the inherent advantages of the batch method, such as the ease of handling, for example. Thus, a skilled person looking for an alternative method to the one disclosed in document D1 and seeking to solve the problem formulated above (and not the more difficult problem, for example, of being able to work with the same efficiency as the method of document D1) would have considered performing the method of document D1 in a batch reaction as an alternative.

17. Consequently, the feature in claim 1 "in a batch reaction" is obviously derivable from a combination of document D1 with the common general knowledge as exemplified by document D15.

The "range" feature

18. As noted above the optimum magnesium ion requirements of the enzymes for transcription and the factors for translation differ. Document D15, describing a linked, cell-free transcription and translation system, performs the transcription reaction at a concentration of 2mM and the translation at 10mM. Document D1 demonstrates that despite the diverging requirements both reactions can be performed at the same time without changing conditions. Thus, even if there should once have existed a prejudice against feasibility of a coupled reaction as alleged by the respondent, at latest with the publication of document D1 it would have been considered baseless.

19. The respondent argues that the claimed magnesium ion ranges are not obvious because in view of document D1 the skilled person would not have had a reason to focus on the magnesium ion concentration since a hint on its importance is missing in the document. The Board disagrees. In order to assess the obviousness of this feature, the question to be answered is not whether it would be obvious in view of the closest prior art document D1. Rather the question is whether, starting from the closest prior art document and having regard to the problem to be solved, the feature would be obviously derivable from **any** available document, including the closest prior art document. Thus, it does not matter if document D1 omits any explicit hint concerning the magnesium ion concentration as long as the relevant information would be derived from other documents. Document D15 is such a document disclosing on page 1926 that "the transcription and translation

reactions are separated temporally because of the disparities among the potassium, magnesium, pH and temperature optima for both reactions." Thus, document D15 contains a hint to optimize the magnesium ion concentration.

20. Additionally, the Board does not share the respondent's view of document D1 not teaching the importance of the magnesium ion concentration. Firstly, the document states in column 3 that "the ratio of the components in the reaction mixture, ion and temperature conditions of the synthesis are optimal for the organisms from which the cell-free systems and exogenous RNA-polymerases are prepared." Since the skilled person reads a document with its background knowledge, represented for example by document D15, he would have inferred from this statement that the reference to "ion" includes magnesium. The skilled person would have found his view supported later in document D1 by the examples where the magnesium concentration is 1,5mM in the case of rabbit reticulocyte lysate and 2,5mM in the case of wheat germ extract. Thus, in the Board's opinion, even without any explicit statement, the skilled person would have recognized the importance of the magnesium ion concentration in view of document D1, so that also for this reason the respondent's argument does not hold.

21. In summary, document D1 conveys the teaching that transcription and translation reactions may be carried out in a coupled manner and that the ion concentrations, inter alia that of magnesium, have to be adapted to the type of RNA polymerase and translation extract or lysate.

22. Thus, the question that remains to be answered with regard to obviousness is whether the ranges provided in claim 1 include what the skilled person would expect should work or whether they are surprising.

23. In the Board's view, the skilled person would, based on its basic knowledge of biochemical reactions, consider it self-evident that the specific magnesium ion concentrations disclosed in document D1 are not the only ones which could possibly be applied. Each biochemical reaction has its optimum conditions. However, around the optimum there is a range of conditions at which the reaction nevertheless proceeds, even though at less than optimal efficiency. This basic knowledge would also make the skilled person confident that in the case of two reactions with differing optimum conditions there would exist an area between the two optima at which both enzymatic reactions can take place. The claimed ranges lie between - and not for example outside - the optimum magnesium ion concentrations for the transcription and translation reaction and therefore meet the expectations of the skilled person.

24. The range of magnesium ion concentrations at which an acceptable reactivity could be achieved was, as submitted during the oral proceedings by the respondent, determined by dilution series and therefore results from routine experiments. Thus, the present case is not one in which an invention is conceived at a theoretical level but where practical realization is difficult and for which the notion of "reasonable expectation of success" was developed in the case law. Rather, the situation is one of "try and see" which the case law

considers to be present if an invention is evident on a theoretical level and can routinely be achieved on a practical level.

25. Hence it is concluded that the feature "wherein said extract is rabbit reticulocyte lysate and the final magnesium concentration is 2.5mM to 3.5mM or wherein said extract is wheat germ extract and the final magnesium concentration is 3.0 mM to 5.25mM" is obvious in view of document D1 in combination with the common general knowledge.

26. Consequently, claim 1 of the main request does not involve an inventive step. Hence, the main request is rejected.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

Registrar: Chair:

C. Eickhoff U. Kinkeldey