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# D E C I S I O N
**of 11 July 2001**

**Case Number:** T 0743/98 - 3.3.4

**Application Number:** 88301112.4

**Publication Number:** 0279582

**IPC:** C12N 15/00

**Language of the proceedings:** EN

**Title of invention:**
DNA sequences to target proteins to the mammary gland for efficient secretion

**Applicant:**
Pharming Intellectual Property BV

**Opponent:**
-

**Headword:**
Target proteins/PHARMING

**Relevant legal provisions:**
EPC Art. 56, 84, 83

**Keyword:**
"Main request - Inventive step (no)"
"Auxiliary request - clarity (no)"
"Sufficiency of disclosure (no)"

**Decisions cited:**
T 0694/92

**Catchword:**
-

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**Case Number:** T 0743/98 - 3.3.4

**D E C I S I O N**
**of the Technical Board of Appeal 3.3.4**
**of 11 July 2001**

**Appellant:** Pharming Intellectual Property BV
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**Representative:** William, Richard Andrew Norman
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**Decision under appeal:** **Decision of the Examining Division of the European Patent Office posted 11 February 1998 refusing European patent application No. 88 301 112.4 pursuant to Article 97(1) EPC.**

**Composition of the Board:**

**Chairman:** U. M. Kinkeldey
**Members:** L. Galligani
S. U. Hoffmann

**Summary of Facts and Submissions**

I. The applicants lodged an appeal against the decision of the examining division issued on 11 February 1998 whereby the application No. 88 301 112.4, filed on 10 February 1988, claiming priority from US 14952 of 17 February 1987, with title "DNA sequences to target proteins to the mammary gland for efficient secretion", was refused pursuant to Article 97(1) EPC.

II. In the view of the examining division the subject-matter of the three requests then on file was new vis-à-vis the following documents:

(D1) EP-A-O 264 166,

(D2) WO-88/00239,

(D3 WO-88/01648,

(D4) Lee, K. F. et al., J. Cell Biol., Vol. 103 (5, Part 2), page 313a, 26th meeting of the American Society for Cell Biology in Washington on 7 to 11 December 1986.

However, it lacked an inventive step having regard in particular to the following publication:

(D5) Clark, A. J. et al., TIBTECH, January 1987, Vol. 5, pages 20 to 24,

in combination with common general knowledge related to the upstream regulatory elements, including the enhancers.

The examining division considered essentially that, since the potential importance of enhancers for eukaryotic gene expression was recognised in the art and the incorporation of a longer stretch of upstream sequences possibly including all the elements necessary for tissue-specific expression was known, there was no inventive merit in merely referring in the claim to enhancer sequences. This was also in view of the fact that the application itself did not particularly emphasise the usefulness of the said sequences and did not identify them in the exemplified constructs. In fact, the teaching of the application did not go beyond what was already obvious from the state of the art.

III. With the statement of grounds of appeal, the appellants submitted the following new document:

(D34) Mercier, J-C., (1986) in "Exploiting New Technologies in Animal Breeding: Genetic Developments", Ed. Smith et al., Chapter 13, pages 122 to 131.

IV. The board issued an official communication with a provisional, non-binding opinion on the issues to be discussed, raising inter alia some objections under Article 123(2) EPC against some of the claims at issue.

V. In reply thereto, the appellants filed on 25 June 2001 an amended main request together with amended first and second auxiliary requests.

Claim 1 of the **main request** (claims 1 to 13) read as follows:

"The use of an enhancer sequence in effecting targeted

mammary gland expression in a transgenic non-human mammal of a coding sequence derived from a gene coding for a biologically active agent, said enhancer sequence being employed, together with said coding sequence, a promoter sequence and a signal sequence in the form of a recombinant DNA gene complex incorporated into the germ line of said mammal, the promoter sequence, enhancer sequence and signal peptide sequence derive from at least one mammary gland-specific gene and facilitate the expression of said coding sequence in the mammary gland."

VI. Oral proceedings took place on 11 July 2001. As a **new auxiliary request**, claims 1 to 13 were submitted in replacement of all auxiliary requests on file. Upon request by the appellants, this request was considered from the perspective of claim 1 as such or with the incorporation of the features of dependent claim 2, or from the perspective of claim 11 alone, with or without the word "optionally".

Claims 1 to 4 and 11 thereof read as follows:

"1. The use of an enhancer sequence in effecting targeted mammary gland expression in a transgenic non-human mammal of a coding sequence derived from a gene coding for a biologically active agent, said enhancer sequence being employed, together with said coding sequence, a promoter sequence, a signal sequence and an intron in the form of a recombinant DNA gene complex incorporated into the germ line of said mammal, the promoter sequence, enhancer sequence and signal peptide sequence derive from at least one mammary gland-specific gene and facilitate the expression of said coding sequence in the mammary gland."

"2. A use as defined in claim 1, wherein said intron links said coding sequence to said promoter or is positioned within said coding sequence"

"11. A process for constructing a recombinant DNA gene complex, comprising adapting said complex to mammary gland targeted expression by linking an enhancer sequence with a promoter sequence, a signal peptide sequence, optionally an intron and a coding sequence, wherein said promoter sequence, enhancer sequence and signal peptide sequence are derived from mammary gland-specific genes, and said coding sequence is a gene coding for a biologically active agent."

VII. In addition to the documents already cited above, the following further documents were discussed:

(D15) Hanahan D., Nature, 9 May 1985, Vol. 315, pages 115 to 122;

(D16) Magram J. et al., Nature, 23 May 1985, Vol. 315, pages 338 to 340;

(D20) Banerji j. et al., Cell, July 1983, Vol. 33, pages 729 to 740;

(D29) Godbout R. et al., Mol. Cell. Biol., February 1986, Vol. 6, No. 2, pages 477 to 487.

VIII. The appellants submitted that the contribution to the art by the application was the identification of the enhancer sequences as essential elements for achieving targeted gene expression in the mammary gland of transgenic non-human mammals. This was reflected by the "use" claims at issue. Nothing in the prior art

suggested or implied such a use.

The closest prior art, both in functional and structural terms, was represented by the disclosure of Lee et al. (D4) which - like the present application - dealt with the problem of how to achieve tissue-specific expression in transgenic mice. However, in their study, the authors investigated only whether there was integration of the genomic clone and whether there was production of mRNA. Lee et al. did not recognise the role of enhancers in effecting targeted mammary gland expression. The low levels of expression observed in just one mouse prompted them to suggest either to insert a genomic clone with more 5'- or 3'-flanking DNA or, as an alternative, to link the 5'-flanking region of the rat ß-casein gene to the CAT (chloramphenicol acetyltransferase) gene. Using their disclosure as a starting point, the skilled person would only have repeated the experiments indicated by the authors. These, however, would not have provided any hint about the role of the enhancers.

None of the other prior art documents referred to the role of enhancers in mammary glands. Although Mercier in (D34) mentioned hybrid gene constructs with the "promoter-enhancer region", he referred to contiguous elements which were used for enhancing gene expression and thus lowering the lactose content in milk, **not** for tissue targeting.

As regards tissue targeting of gene expression in transgenic mice, the available prior art documents referred only to the inclusion in the constructs of 5'-flanking and/or 3'-flanking DNA sequences as these were known to contain regulatory elements, no mention being

made of enhancers.

The few prior art documents, which mentioned enhancers - like (D20) and (D29) - dealt exclusively with cell-specific expression in model systems, **not** with tissue-specific expression in vivo. These documents would have neither suggested a role of enhancers in vivo, nor provided a reasonable expectation that enhancers could have a tissue-specific effect in transgenic animals.

The present patent application contributed to the art in vivo experiments in eight mice that demonstrated the role of the enhancers in targeting expression in mammary glands (cf Figures 6 and 7). The application also indicated how, by using the enhancer trap assay, the skilled person could screen for tissue-specific enhancers. The disclosure had thus converted any possible speculation and/or hopes of the prior art about the function of enhancers in the concrete expectation of a role in vivo. Thus, differently from the case of T 694/92 (Modifying plant cells/MYCOGEN, OJ EPO 1997, 408) where doubts remained about the feasibility of the method claimed in large areas of the claims (eg monocotyledonous plants), the claimed use was fully supported by the disclosure in its broad outline and there was no real combination of prior art documents which could have made it obvious to the skilled person.

IX. The appellants requested that the decision under appeal be set aside and a patent be granted on the basis of either the main request or the auxiliary request. In writing they also requested that the appeal fee be refunded for serious abuse of the procedure by the examining division.

## Reasons for the Decision

*The main request*

1. The board has no formal objections under Article 123(2) EPC against any of the claims of this request. The use of enhancer sequences linked to promoter and signal sequences, all being derived from at least one mammary gland-specific gene, in recombinant DNA constructs to be targeted to mammary glands in transgenic non-human mammals is supported in particular by pages 4 to 6 of the application as filed.

2. The novelty of the claimed subject-matter over (D1) to (D4), which was acknowledged by the examining division, is not questioned by the board as indeed none of the said documents specifically discloses the use of enhancers in recombinant DNA constructs for gene targeting in mammary glands of transgenic mammals.

3. The remaining issue to be discussed is that of inventive step. The examining division, in its decision, expressed the view that the technical teaching of the application was scanty and did not go beyond what was already obvious from the state of the art.

4. The assessment of inventive step might require balancing the contribution to the art by the patent specification from the intellectual point of view (ie the idea underlying the claimed invention) against the actual technical disclosure provided in support of what is claimed (ie the extent and sufficiency of the

description). Such an exercise is often necessary in cases where, for example, the contribution to the art by a patent application is the demonstration that something which was already theoretically conceivable based on the prior art is indeed feasible, and the specification, apart from the experimental verification, does not provide additional technical details in comparison with the prior art (cf case of T 694/92 supra). In such cases, claims with a broad outline may be found either to lack an inventive step or to relate to subject-matter which is not sufficiently disclosed.

5. In the present case, in agreement with the appellants, the board considers that the closest prior art is represented by the disclosure of Lee et al. (D4) which describes experimental work aimed at achieving in transgenic mice tissue-specific expression of the rat ß-casein gene. To this extent, they introduced into mouse embryos a genomic clone containing the entire gene with 1.3kb of 5'-flanking DNA and 0.4kb of 3'-flanking DNA. One positive transgenic mouse showed integration of the construct which was also inherited by the F1 offspring. The ß-casein gene was indeed expressed, although at low levels, in the lactating mammary gland. The low expression levels were considered to be the result of either "insufficient" flanking DNA being used or of the presence of prokaryotic DNA or of the site of integration. To test this, the authors proposed either to use a genomic clone containing more flanking DNA, namely 3.5kb of 5'-flanking DNA and 3.0kb of 3'-flanking DNA and no prokaryotic DNA, or, as an alternative, to link the 5' flanking region of the gene to the CAT gene and determine if CAT expression was selectively targeted to

the mammary gland.

6. In the light of the said disclosure, the technical problem underlying the present application was finding an alternative way for effecting targeted mammary gland expression in transgenic animals, in particular in transgenic mice.

7. As a solution, claim 1 proposes using an enhancer sequence together with a promoter and a signal sequence, all being derived from at least one mammary gland-specific gene, in recombinant DNA constructs containing a DNA sequence to be targeted.

8. The experimental part of the application shows that no enhancer sequence was specifically isolated or identified and used as such for targeting. Enhancer sequences were used as part of larger 5'- or 3'-flanking sequences. In the working example in which tissue-specific gene expression was tested, the enhancer sequence was within a genomic clone containing the entire rat ß-casein gene with larger 5' and 3' flanking sequences, namely 3.5kb of 5'-flanking DNA and 3.0kb of 3'-flanking DNA, prokaryotic DNA being eliminated. As a matter of fact, this example is precisely the experiment suggested by Lee et al. in (D4) for achieving higher levels of expression (cf point 5 above). The present specification shows that thereby targeted ß-casein expression was achieved in the lactating mammary gland of eight mice (cf. column 19 and Figures 6 and 7 of the published patent application).

In respect of finding tissue-specific enhancer sequences present within a gene, the specification

refers, without directly mentioning it, to the so-called "enhancer-trap assay" (cf column 10, lines 34 to 47 of the published application), no examples being given of the isolation and characterisation of particular enhancer sequences.

9. The relevant question in relation to inventive step is whether the skilled person, faced with the technical problem as stated above, would have readily adopted the measure of using 5'- or 3'- flanking sequences large enough to **knowingly** include an enhancer.

10. As stated in the declarations of Dr Peter Gruss and of Dr Rudolf Grosschedl filed during the prosecution before the examining division, in early '87 "enhancers" were recognised to be regulatory elements distinct from promoters which, without regard to their position or orientation with respect to the coding DNA, stimulated transcription and could in some cases be located also far away from the transcription unit.

11. As regards the issue of targeting DNA expression to particular cells or tissues, in early '87 the knowledge had already emerged that for it to be achieved large enough 5'-flanking DNA and/or 3-'flanking DNA had to be included in the constructs, because - as explained, for example, by Clark et al. in their review Article (D5), cf right column on page 21 - "*with short segments, comprising only the promoter, the coding sequence and a few hundred nucleotides at either side, tissue-specific expression often becomes dependent upon the precise site of integration, and only a proportion of transgenic progeny express the transgene appropriately*". In early '87, there was also an increased awareness of the importance of including the

multiple and functionally distinct regulatory elements in order to target the expression to specific cells or tissues (cf eg Clark et al., loc. cit. left column on page 22; Godnout et al. (D29); Hanahan (D15); Magram et al. (D16)). In this context, "enhancers" were also known to participate in cell and tissue specificity (cf Godnout et al. loc. cit., page 485, left-hand column, last paragraph; and Hanahan, loc.cit. Figure 1 and page 118, right-hand column, last paragraph; Banerji et al. (D20), abstract).

12. As regards the issue of targeting DNA expression to the mammary glands of transgenic animals, the following was prior art:

(a) A review article by Clark et al (D5) had indicated that by combining regulatory elements derived from one gene with the coding sequence of another it was possible to direct the synthesis of a particular protein to a specific body tissue, and had thereby made particular reference to mammary glands as a possible target in order to harvest proteins from the milk of transgenic dairy animals;

(b) Mercier (D34) in his outline of the prospects for genetic engineering applied to milk producing animals had suggested, as a way to lower lactose content of milk by means of germ line manipulation, the injection into the pronuclei of animal eggs of a construct in which a hybrid ß-galactosidase gene containing the signal peptide of a milk protein (eg a casein or ß-lactoglobulin) was fused to the **promoter-enhancer** region of a milk protein gene.

13. In the board's judgement, the skilled person, starting from the disclosure by Lee et al. (D4) (cf point 5 above), and faced with problem of finding an alternative way for effecting targeted mammary gland expression in transgenic animals (cf point 6 above), would have readily followed the suggestion of Lee et al. to include more 5' and 3' flanking DNA, because this was fully in line with the prior art suggesting such a measure in order to achieve effective targeting (cf point 11 above). In taking this approach, the skilled person, being aware that enhancer sequences participated together with other regulatory elements in cell and tissue specificity (cf point 11 above), would have **knowingly** included them in the recombinant DNA constructs, also in consideration of the fact that in document (D34), a textbook reference which the skilled person would not have overlooked, constructs with promoter-enhancer were proposed in the context of targeted expression.

14. The arguments put forward by the appellants that Mercier (D34) (a) dealt with the specific problem of reducing the lactose content in milk, (b) referred to contiguous "enhancer-promoter" regions, and (c) was not addressing the problem of targeting, are not convincing for the reasons that: (i) claim 1 at issue excludes neither targeting ß-galactosidase to mammary glands nor the use of contiguous "promoter" - "enhancer" elements, and (ii) targeting was the issue also in (D34) as the goal was to achieve the presence of ß-galactosidase in milk, which implied targeting the expression of the hybrid gene in mammary glands where milk is produced.

15. As for the appellants' argument that the use of enhancers in the prior art was limited to testing cell-

specific expression in model systems, **not** tissue-specific expression in vivo, and that this would have neither suggested a role of enhancers in vivo, nor provided a reasonable expectation that they could have a tissue-specific effect in transgenic animals, the board does not see the prior art indications as being so strictly limited to in vitro model systems. In fact, Hanahan (D15), for example, reported experiments in which the targeting of DNA expression to the pancreas of transgenic mice was mediated by the insulin control region containing an enhancer element (Figure 1 and page 118, right-hand column, last paragraph). Moreover, document (D29), in the discussion, referred to the enhancers as contributing to tissue-specificity (page 485, left-hand column, last paragraph).

16. As for the skilled person's expectations in respect of the role of enhancers in mammary glands, these would not have been negative in view of the fact that prior art was rather encouraging as regards generally the role of enhancers in the determination of cell and tissue specificity. There were no reasons for the skilled person to think that mammary tissue would be a problematic area. On the contrary, Mercier (D34) had referred to enhancers in the context of targeting gene expression to milk-producing tissue (cf point 12, item b) above).

17. As already noted above, the specification does not conclusively demonstrate a role of enhancers as such, ie in isolated form, in targeting gene expression. It rather shows a role of enhancers as part of larger 5'- or 3'-flanking DNA. For the reasons given above, the skilled person would have readily derived from the prior art the idea of using larger flanking sequences

including an enhancer sequence in order to target expression in mammary glands. Indeed, it can be agreed with the examining division that the specification contributed to the art essentially only the experimental demonstration that what was obviously conceivable on the basis of the prior art was feasible (cf point 4 above). Consequently, the subject-matter of claim 1 is considered to be obvious to the skilled person, and the request of which it is part is not allowable under Article 56 EPC.

*The auxiliary request*

18. In independent claims 1 and 11 of this request, the feature "an intron" is contained, its nature and positioning in respect to the other elements (enhancer, promoter, signal and coding sequences) not being specified. Claim 2 indicates that the said intron either links the coding sequence to the promoter or is positioned within the coding sequence. Formal support for the feature in the context of the claims is found on page 10, lines 23 to 27 and in Figure 8 of the application as filed so that no objections under Article 123(2) EPC arise.

19. When asked about the relevance of such a feature, the appellants made reference to the submissions in a letter dated 14 April 1993 to the examining division, where it was indicated that the two constructs ß -511/+535 and ß -2300/+535 of Figure 8 contained a so-called cryptic acceptor site from which splicing could occur notwithstanding the absence of the normal acceptor site, and that constructs in which all intron sequences were deleted showed very much reduced expression. This, in their view, showed that introns

increased the efficiency of expression.

20. Apart from the fact that such findings are related to some specific constructs and no general conclusion is possible therefrom, the said information is not part of the original disclosure and cannot be derived from it. Thus, it was not available to the skilled person.

21. The specification is completely silent about the function of said intron sequence as well as about where from the intron should be derived, so that the intron could in fact be derived from anywhere and have nothing to do with the coding DNA. Introns, being regions of a gene which lie between exons and do not code for a translated product, have to be spliced out after transcription. This does not occur if correct splicing sequences are not included, and in such a case the desired protein is not produced. No guidance at all is provided by the application in this respect. Nor it is specified what the feature "positioned within the coding sequence" (cf claim 2) means (genomic clone? artificial construct of homologous or heterologous elements?).

22. Under these circumstances, the board considers that claim 1 of the auxiliary request, when considered per se or in combination with the features of claim 2, and claim 11 with or without the word "optionally" do not define in clear technical terms what is claimed (objection under Article 84 EPC), and that the description of the specification is not sufficiently clear and complete for the skilled person to perform what is claimed (objection under Article 83 EPC).

23. For these reasons, the auxiliary request is not

allowable.

*Other matters*

24. Rule 67 EPC provides for the possibility of reimbursement of the appeal fee "where the Board of Appeal deems an appeal to be allowable". In the present case, as the appeal is dismissed, the first condition for the reimbursement of the appeal fee, which had been requested in writing by the appellants, is not fulfilled.

## Order

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

The appeal is dismissed.

The Registrar: The Chairperson:

N. Maslin U. Kinkeldey