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**Datasheet for the decision  
of 20 October 2016**

**Case Number:** T 1285/13 - 3.3.08

**Application Number:** 02782801.1

**Publication Number:** 1430128

**IPC:** C12N15/11, A61K48/00

**Language of the proceedings:** EN

**Title of invention:**

MICRO-RNA MOLECULES

**Applicant:**

Max-Planck-Gesellschaft zur Förderung  
der Wissenschaften e.V.

**Headword:**

miR-1/MAX-PLANCK-GESELLSCHAFT

**Relevant legal provisions:**

EPC Art. 123(2), 54, 56

**Keyword:**

Main request - requirements of the EPC met (yes)

**Decisions cited:**

T 1329/04, T 0898/05

**Catchword:**



**Beschwerdekammern**  
**Boards of Appeal**  
**Chambres de recours**

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Case Number: T 1285/13 - 3.3.08

**D E C I S I O N**  
**of Technical Board of Appeal 3.3.08**  
**of 20 October 2016**

**Appellant:** Max-Planck-Gesellschaft zur Förderung  
(Applicant) der Wissenschaften e.V.  
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**Representative:** Jellbauer, Stephan  
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**Decision under appeal:** Decision of the Examining Division of the  
European Patent Office posted on 19 November  
2012 refusing European patent application No.  
02782801.1 pursuant to Article 97(2) EPC.

**Composition of the Board:**

**Chairman** M. Wieser  
**Members:** B. Stolz  
D. Rogers

## **Summary of Facts and Submissions**

- I. The applicant (appellant) filed an appeal against the decision of the examining division whereby the European patent application No. 02 782 801.1 was refused. The examining division decided that the main request submitted with letter of 8 June 2012 did not meet the requirements of Articles 123(2), 56 and 57 EPC, and that the auxiliary request submitted at the same date did not meet the requirements of Articles 56 and 57 EPC.
- II. With its statement setting out the grounds of appeal, the appellant maintained auxiliary request I and submitted a new main request and a new auxiliary request II.
- III. The appellant was summoned to oral proceedings. A communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) annexed to the summons, informed it of the preliminary non-binding opinion of the board on some of the issues to be discussed at the appeal proceedings.
- IV. Oral proceedings were held on 20 October 2016. At the onset of the oral proceedings the appellant replaced its auxiliary requests I and II by corrected versions. In the course of these proceedings, the appellant withdrew its main request and made corrected auxiliary request I, filed at the oral proceedings, its new main request.
- V. Claim 1 of the main request reads as follows:  
  
"1. An isolated nucleic acid molecule having a length of from 18 to 25 nucleotides comprising a

nucleotide sequence which has an identity of at least 90% to a sequence shown in SEQ ID NO 58 (miR-1) or a complement thereof."

Dependent claims 2 to 13 define specific embodiments of the subject matter of claim 1. Claim 14 defines a recombinant expression vector comprising a nucleic acid molecule of claim 1.

VI. The following documents are referred to in this decision:

D1: PASQUINELLI AMY E. et al., NATURE vol. 408, no. 6808, 2 November 2000, pages 86-89;

D2: CALIN GEORGE A. et al., PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 99, no. 24, 26 November 2002, pages 15524-15529;

D10: KWON CHULAN et al., PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 102, no. 52, December 2005, pages 18986-18991;

D11: ZHAO YONG et al., NATURE (LONDON), vol. 436, no. 7048, July 2005, pages 214-220;

D12: ZHAO YONG et al., CELL, vol. 129, no. 2, April 2007, pages 303-317;

D14: ELBASHIR SAYDA M. et al., GENES and DEVELOPMENT, vol. 15, 15 January 2001, pages 188-200.

VII. The arguments of the appellant can be summarized as follows:

Article 56 EPC

Document D1, representing the closest state of the art, described two small regulatory RNA molecules, lin-4 and let-7. The technical problem consisted in finding additional regulatory RNA molecules of this type, i.e. physiological regulatory miRNAs. The molecule of claim 1, miR-1 possessed a number of characteristic properties of this class of regulatory molecules. According to document D1, these properties included a size of 21 or 22 nucleotides conserved across phylogeny, a precursor structure with the potential to form a stable stem-loop structure, a pattern of temporal regulation of expression and a mechanism of action involving binding to complementary sites in the untranslated regions of genes. miR-1 had the right size, a precursor molecule capable of forming a stable stem-loop structure, it was conserved among different species and showed temporal regulation during Drosophila development. These results led to the conclusion that miR-1 was a physiological regulatory molecule, i.e. a solution to the underlying technical problem. This conclusion was confirmed by post-published documents D2 to D12. Importantly, there was no evidence to the contrary on file.

Document D1 stated that further miRNAs might exist but disclosed no means how to identify any. Nor did the remaining prior art suggest any methods for this purpose. The subject matter of claim 1 was therefore inventive.

Article 57 EPC

The assignment of miR-1 to the class of miRNAs was not arbitrary but based on a number of structural and functional properties which it shared with lin-4 and let-7. These included its size, a precursor molecule having the potential to form stable stem-loop structures, the fact that it was encoded in multiple species, and Table 1 clearly showed that it was differentially expressed during the development of *Drosophila* embryos. The application suggested that miR-1 was useful for the modulation of gene expression. Document D10 confirmed this conclusion. The expression pattern of miR-1 showed that it was useful for the classification of developmental stages of *Drosophila*. This in itself represented an immediate concrete benefit and provided industrial applicability as formulated in decision T 898/05 of 7 July 2006.

- VIII. The final request of the appellant was that the decision under appeal be set aside and that a patent be granted on the basis of the Main Request submitted at the oral proceedings before the Board.

**Reasons for the Decision**

Article 123(2) EPC

1. The main request differs from auxiliary request I (filed on 8 June 2012) underlying the decision under appeal only by amended back references of dependent claims 6 to 13. These amendments merely adapt incorrect back references of claims 6 to 13 of the earlier auxiliary request I and do not introduce new subject matter.

The requirements of Article 123(2) EPC are met.

#### Priority right

2. In its preliminary opinion attached to the summons to oral proceedings (cf. point 21), the board noted that the patent application contains a reference to a document, "[37]", *Lee and Ambros*, 26 October 2001, *Science* 294, 862-864, which disclosed the nucleic acid sequence of the miR-1 molecule of the present application and its expression in human heart cells. The publication date of this document was shortly after the first priority date and before the filing date of the second priority application of the present patent application. Thus, this document became prior art under Article 54(2) EPC for subject matter of the present application which was directly and unambiguously disclosed only after the first priority date (i.e. in the second priority application or later). A side by side comparison of the patent application and the first priority application showed that this concerns Example 2 and Figures 5, 6 and 7 of the patent application. The contents of the description of the first priority application have been incorporated into the patent application in their entirety.

It follows that only subject matter which is directly and unambiguously disclosed in the first priority application is not affected by the disclosure of *Lee and Ambros*.

3. Nucleic acid molecule miR-1 as defined by Seq ID NO: 58 is disclosed in Tables 1 and 2 of the first priority document. The subject matter of claim 1 of the main request is disclosed in claim 2 (by reference to Table



2) of the first priority document. The subject matter of dependent claims 2 to 13 and independent claim 14 is directly and unambiguously disclosed by claim 5 via reference to the preceding claims 3 and 4, claims 8 to 12, and pages 3 (in particular lines 14, 26 and 28) and 4 (in particular line 6) of the description of the first priority document. Thus, the subject-matter of claims 1 to 14 of the main request is entitled to the first priority date and *Lee and Ambros* do not form part of the state of the art.

Article 54 EPC

4. There is no prior art on file anticipating the subject matter of the main request which, accordingly, meets the requirements of Article 54 EPC.

Article 56 EPC

5. Document D1, representing the closest prior art, discloses two small RNAs (termed stRNAs for small temporal RNAs), *lin-4* and *let-7*, both having a role in the timing of the development of the nematode *C. elegans*. *let-7* RNAs of about 21 nucleotides in length were detected in samples from a wide range of animal species including vertebrates and many others (cf. abstract and Figure 2) and its role in the temporal regulation of physiological processes was found to be conserved in *C. elegans*, *Drosophila*, zebrafish, annelids and molluscs. Three exact homologs and two imperfect homologs matching 20/21 nucleotides of *let-7* were detected in humans. Similar stem-loop secondary structures were predicted for precursor transcripts of *C. elegans*, *Drosophila*, and human *let-7* RNAs (cf. Figure 1). The expression levels of the human *let-7* RNA varied among tissues, indicating possible cell-type regulation

of let-7 expression (page 87, left column). Although vertebrates do not develop through larval stages, expression of let-7 in Zebrafish was also temporally regulated (cf. Figure 3b). From these data, the authors concluded that although there was no proof that let-7 homologues across phylogeny were temporally regulated, the evidence in support of a conserved function was strong, because of the conservation of sequence, of a longer structured precursor, of the 21 nucleotide length, of temporal regulation and of complementary target sites in a particular gene (lin-41) in *C. elegans*, *Drosophila* and Zebrafish (cf. Figure 1b). The highly conserved length of the let-7 RNA was taken as an indicator that length was central to the performance of its function. The authors therefore proposed that let-7 RNA was likely to regulate developmental timing in bilaterian animals.

6. The examining division initially defined the technical problem to be solved, when starting from document D1, as the provision of a diagnostic or therapeutic substance for a medical condition such as heart disease (cf. point 16 of the decision under appeal).
  
7. The examining division decided that the claims before it lacked an inventive step because the application did not credibly or plausibly show that miR-1 indeed was a regulatory RNA molecule. It considered that neither the size of the molecule nor the fact that it showed a certain degree of conservation across species allowed a conclusion about a possible role of the molecule (cf. point 11 of the decision under appeal). It also considered that the application neither demonstrated nor hypothesized about a specific regulatory role or a target of the of miR-1 molecule (cf. points 13 and 14). It was therefore decided that the technical problem as

defined in point 6, supra, was not credibly solved and the problem underlying the claimed invention was redefined as the provision of a further nucleic acid sequence without known function. The claimed solution to this problem, miR-1, was found to be arbitrary and not to involve an inventive step (cf. point 17 of the decision under appeal).

8. The board agrees with the examining division's conclusion in so far as the definition of the technical problem as stated in point 6, above, was too ambitious. Indeed the application provides no evidence for a target gene regulated by miR-1.
9. The board, however, considers that the available technical information allows the formulation of a different technical problem, which is more ambitious than the alternative technical problem defined by the examining division. This problem is defined as the provision of a further small physiological regulatory RNA molecule.
10. According to the established jurisprudence of the boards of appeal the assessment of inventive step is to be made at the effective date of the patent on the basis of the information in the patent together with the common general knowledge then available to the skilled person. The verification of whether or not the claimed solution actually solves the problem, i.e. whether the claimed subject-matter actually provides the desired effect, must be based on the data in the application. Post-published evidence to support that the claimed subject-matter solves the underlying technical problem may be taken into account if it is already credible from the disclosure in the patent that

the problem is indeed solved. (cf. Case Law of the Boards of Appeal, 8th edition, 2016, I.D.4.6., p. 181).

11. Applied to the present case, this means that the definition of the invention as being a contribution to the art, i.e. as solving a technical problem and not merely putting forward one, requires that it is at least made plausible by the disclosure in the application that miR-1 indeed is a physiological regulatory molecule. If this is the case, the supplementary post-published evidence submitted by the appellant as documents D2 to D13 may be taken into consideration (cf. point 12 of decision T 1329/04 of 28 June 2005).
  
12. It needs therefore to be assessed whether the available evidence supports and makes plausible a role of miR-1 as a regulatory RNA molecule.

Similarities of miR-1 with lin-4 and let-7:

13. Seq ID No 58 defines miR-1 as an RNA of 22 nucleotides in length. According to page 3, lines 13 and 14, of the first priority document, mature miRNAs usually have a length of 19 to 24 nucleotides, particularly 21, 22 or 23 nucleotides. The size of 22 nucleotides is very similar to the 21 nucleotides of let-7 disclosed in document D1. miR-1 molecules of this size were cloned from isolated, size fractionated RNAs of *Drosophila* embryo lysates (Table 1), and HeLa cell (human) total RNA (Table 2). They are thus real and not merely predicted micro RNAs.

According to page 3, lines 21 to 24, of the first priority document, an miRNA is usually a single stranded molecule, while its precursor molecule is

usually an at least partially self-complementary molecule capable of forming double stranded portions, e.g. stem- and loop-structures. As explained on page 14, lines 3 to 5 of the first priority application, a database search revealed that the miR-1 sequence is indeed flanked by sequences with the potential to form a stable stem-loop structure. The predicted stem-loop structure is shown in Figure 3. In terms of secondary structure, this predicted structure looks very similar to the precursor structure predicted for let-7 (cf. Fig 3 of both, the patent and the first priority application and Figure 1 of document D1).

Thus, at the structural level, miR-1 shares important properties with let-7.

14. As for possible functions, the priority application describes the new miRNAs as "*molecules associated with physiological regulatory mechanisms*" (page 1, lines 1 to 3).
15. According to document D1 (cf. abstract), lin-4 and let-7 regulate the timing of *C. elegans* development.
16. Table 1 shows that miR-1 is differentially expressed during the development of *Drosophila* embryos with the highest expression levels in larval stages L1 to L3 and in adult flies. The first priority application states in this respect: "*The temporal expression of miR-1, miR-2 and miR-8 to miR-13 was less restricted. These miRNAs were observed at all developmental stages though significant variations in the expression levels were sometimes observed was less restricted.*" (page 11, lines 28 to 31). According to page 12, lines 3 to 6, this expression pattern is very similar to that of the lin-4 stRNA disclosed in document D1.

17. Further, according to document D1, the fact that let-7 is highly conserved across phylogeny is indicative of a conserved function.
18. In this respect, the first priority document discloses (page 13, lines 1 to 11) that Northern blots show the existence of miR-1 in *C. elegans*, *C. briggsae*, zebrafish, mouse and cow. Interestingly, although it could be isolated from HeLa cell RNA extracts, miR-1 could not be detected by Northern blots of HeLa cells. According to page 13, lines 8 to 11, "*This represents another case of tissue-specific expression of a miRNA, and indicates that miRNAs may not only play a role in developmental timing, but also in tissue specification.*"
19. Thus also at the functional level, miR-1 shares important properties of a developmental regulator with lin-4 and let-7.
20. As a consequence, the board is convinced that the combined structural and functional information presented in the first priority application renders the claimed role for miR-1 in the temporal and tissue specific gene regulation plausible and credible.
21. As for a potential mechanism of action, the priority application states that "*the claimed molecules may be used as a modulator of the expression of genes which are at least partially complementary to said nucleic acid*" (page 5, lines 27 to 30).
22. Post-published evidence confirms that miRNAs play a role in the temporal and tissue specific regulation of genes (e.g. document D2) and document D10 provides

experimental evidence that miR-1 indeed modulates cardiogenesis in *Drosophila*. It describes that putative miR-1 binding sites were found in the 3'-UTR of the gene encoding "Delta" (page 18989, right column, second but last paragraph), thereby confirming the postulated mechanism of action. Documents D11 and D12 demonstrate an effect of the corresponding miR-1 homologue in the cardiogenesis of mice.

23. In view of the fact that the technical information disclosed in the first priority document renders a physiological regulatory role of miR-1 credible, that there is no evidence on file showing that the conclusions drawn on the basis of this technical information are incorrect or based on the wrong assumptions, and that the putative role assigned on the basis of the disclosed technical information is confirmed by post-published evidence, the board concludes that the technical problem of providing a further physiological regulatory molecule is indeed solved by the nucleic acid molecule of claim 1.
24. It remains to be established whether the claimed solution involves an inventive step.
25. At the filing date of the first priority application, *lin-4* and *let-7* RNAs were the only two small expressed RNAs known to have a function as regulators of developmental timing. Document D1 suggests that "*Genome sequence comparisons and expression analyses among bilaterian animals may reveal additional stRNAs that regulate other developmental transitions*" (page 88, final sentence). Document D1 does, however, not suggest or otherwise point to a way of finding any additional micro RNA sequences. The general reference to sequence comparisons and expression analyses is unspecific and

does not render obvious how the skilled person would identify additional miRNAs with a physiological regulatory role with a reasonable expectation of success. Based on the disclosure of document D1 alone, the claimed solution is therefore not obvious.

26. As described in Example 1 of the priority application, the applicant used a procedure for the isolation of short RNAs from several organisms and cell types which it originally developed to study the mechanism of RNA interference. The original method was described in Document D14 (cited as reference [8] in the patent application) and published before the filing date of the first priority application.
27. According to document D14, the natural function of RNA interference appeared to be the protection of the *Drosophila* genome against invasion by mobile genetic elements (page 188, left column, second paragraph). Document D14 describes experiments in which double stranded RNAs of various lengths were added to *Drosophila* embryo extracts. The RNAs added to these extracts were processed to shorter RNAs of 21 or 22 nucleotides in length and subsequently isolated by a particular cloning procedure involving size fractionation followed by ligation of 5' and 3' adaptor molecules. The cloned short RNA molecules were then sequenced in order to further elucidate the mechanism of RNA interference. Nothing in this document points to the existence of 21 nucleotide short RNAs other than the interfering RNAs derived from the externally added double stranded RNA molecules.
28. At the filing date of the first priority document, there was no evidence that cellular extracts provided a suitable source of sufficiently small regulatory RNA



molecules. Even if the skilled person was aware of document D14, it would therefore not have considered to apply the cloning procedure disclosed therein to cellular extracts in order to solve the above mentioned technical problem with a reasonable expectation of success.

29. The board decides therefore that the subject matter of claims 1 to 14 involves an inventive step and meets the requirements of Article 56 EPC.

Article 57 EPC

30. The examining division decided that the requests before it not only lacked an inventive step but also industrial applicability. The examining division decided that the indication of achievable objectives given in the application did not go beyond speculation and vague general statements. Therefore, no defined industrial application was considered to be disclosed.
31. The issue of Article 57 EPC is closely related to the question whether or not the technical problem underlying the invention has been credibly solved. As shown in points 13 to 23 above, the board is convinced that the priority application plausibly discloses a role for miR-1 in the regulation of physiological processes. The application predicts a role in developmental timing by mediating sequence specific repression of RNA translation (page 1, lines 20-22). This prediction is supported by the data of example 1, Table 1 and Figure 1a) which show expression of miR-1 in a developmental stage specific manner in *Drosophila*. This function is confirmed by later published document D10 which shows that miR-1 influences cardiac development. The priority application provides also

evidence of a conserved function across different species and of tissue specific expression.

32. Lin-4 and let-7, the two stRNAs disclosed in the prior art, were both known as regulators of developmental timing (cf. document D1). It is on this basis that the role of miR-1 as a developmental regulator was assigned. Neither the prior art nor the post-published documents provide any evidence for a different function. The role as a regulatory molecule of developmental timing is specific enough to be of immediate concrete benefit in the sense of point 6 of the reasons of decision T 898/05 of 7 July 2006. At the least miR-1 may be used for the staging of development in *Drosophila*. Furthermore, in view of its presence in various species it may be used for the modulation of development which is of importance to industry in view of developmental dysfunctions such as cancer (cf. page 5, lines 18 to 20 of the priority application).
33. The main request therefore meets the requirements of Article 57 EPC.

## Order

### For these reasons it is decided that:

1. The decision under appeal is set aside.
2. The case is remitted to the department of first instance with the order to grant a patent on the basis of the Main Request submitted at the oral proceedings before the Board on 20 October 2016 and a description to be adapted.

The Registrar:

The Chairman:



A. Wolinski

M. Wieser

Decision electronically authenticated