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Case Number: T 1068/07 - 3.3.08

Application Number: 98920015.9

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Language of the proceedings: EN

Title of invention:
Enzymatic DNA molecules

Applicant:
THE SCRIPPS RESEARCH INSTITUTE

Headword:
Enzymatic DNA/SCRIPPS II

Relevant legal provisions:
EPC Art. 123(2)

Keyword:
"Main request - added subject-matter (yes)"

Decisions cited:
T 1068/07, G 0002/10

Catchword:
In line with the decision G 2/10 of the Enlarged Board of Appeal, for a disclaimer that diclaims from a claim subject-matter disclosed in the application as filed to fulfil the requirements of Article 123(2) EPC, the subject-matter of the disclaimer introduced in that claim has to be - either explicitly of implicitly - directly and unambiguously disclosed in the application as filed, and the subject-matter remaining in this claim after the introduciton of the disclaimer in this claim has to be - either explicitly or

implicitly - directly and unambiguously derivable from the application as filed (see points 1 and 2 of the Reasons for the decision).

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 1068/07 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 7 March 2012

Appellant: THE SCRIPPS RESEARCH INSTITUTE
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted on 2 February 2007 refusing European patent application No. 98920015.9 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman: M. Wieser
Members: P. Julià
D. S. Rogers

Summary of Facts and Submissions

I. European patent application No. 98920015.9, published as International patent application WO 98/49346 (hereinafter "*the application as filed*") was refused by the examining division. In its decision of 2 February 2007, the examining division considered claim 1 of the Main Request and of the Auxiliary Request I not to fulfil the requirements of Article 123(2) EPC because, in its view, there was no basis in the application as filed for the disclaimers introduced in claim 1 of these Requests. Moreover, according to the examining division, these disclaimers did not meet the criteria laid down by the Enlarged Board of Appeal in the decision G 1/03 (OJ EPO 2004, 413) since the prior art document D1 (WO 96/17086) disclosed the subject-matter of these disclaimers and it was not so unrelated and remote from the claimed invention to be considered as an accidental anticipation.

II. The applicant (appellant) lodged an appeal against this decision. On 25 June 2010, oral proceedings were held before the board, after a communication of the board pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) and the appellant's reply thereto filed on 25 May 2010 and containing Auxiliary Requests II and III. At the end of the oral proceedings, the following question was referred *ex officio* by the board to the Enlarged Board of Appeal:

"Does a disclaimer infringe Article 123(2) EPC if its subject-matter was disclosed as an embodiment of the invention in the application as filed?" (cf. OJ EPO 2011, page 256).

III. In its decision G 2/10 of 30 August 2011 (to be published in the OJ EPO), the Enlarged Board of Appeal replied to the above question stating that:

"1a. An amendment to a claim by the introduction of a disclaimer disclaiming from it subject-matter disclosed in the application as filed infringes Article 123(2) EPC if the subject-matter remaining in the claim after the introduction of the disclaimer is not, be it explicitly or implicitly, directly and unambiguously disclosed to the skilled person using common general knowledge, in the application as filed.

1b. Determining whether or not that is the case requires a technical assessment of the overall technical circumstances of the individual case under consideration, taking into account the nature and extent of the disclosure in the application as filed, the nature and extent of the disclaimed subject-matter and its relationship with the subject-matter remaining in the claim after the amendment."

IV. On 23 September 2011, the board requested the appellant to clarify its requests in view of the decision G 2/10 (*supra*).

V. In its reply of 5 December 2011, the appellant withdrew its Main Request and Auxiliary Request I then on file. Previous Auxiliary Requests II and III, both filed on 25 May 2010 in reply to the board's communication under Article 15(1) RPBA (cf. Section II *supra*), were maintained as Main Request and Auxiliary Request I, respectively.

VI. Appellant's **Main Request** consisted of 46 claims, wherein claim 1 read as follows:

"1. A catalytic DNA molecule having site-specific endonuclease activity specific for a nucleotide sequence defining a cleavage site in a preselected substrate nucleic acid sequence,
said catalytic molecule having first and second substrate binding regions flanking a core region,
said molecule having the formula:

5' (X-R) - GGCTAGCT^{8}ACAACGA - (X) 3'

wherein

each X is any nucleotide sequence,
(X-R) represents said first substrate binding region,
(X) represents said second substrate binding region,
R is a nucleotide capable of forming a base pair with a pyrimidine in the preselected substrate nucleic acid sequence,
T^{8} may be replaced by C or A,

said first substrate binding region having a sequence capable of binding through complementary base-pairing to a first portion of said preselected substrate nucleic acid sequence,
said second substrate binding region having a sequence capable of binding through complementary base-pairing to a second portion of said preselected substrate nucleic acid sequence,

with the proviso that said catalytic molecule is not a molecule in which the first and second binding regions can bind through complementary base-pairing to a substrate nucleic acid which is

5' - GGAAAAAGUAACUAGAGAUGGAAG - 3' (SEQ ID NO 135)."

Claims 2 to 23 were directed to specific embodiments of claim 1. Claims 24 and 25 related to a composition comprising two or more populations of catalytic DNA molecules according to claim 1, wherein each population of catalytic DNA molecules was capable of either cleaving a different nucleotide sequence in a substrate (claim 24) or of recognizing a different substrate (claim 25). Claims 26 was directed to a method of cleaving a target nucleic acid molecule and claim 30, containing the same disclaimer as claim 1, to a method of engineering a catalytic DNA molecule. Claims 27 to 29 and claims 31 to 46 were specific embodiments of claims 26 and 30, respectively.

VII. The arguments of the appellant, insofar as they are relevant to the present decision, may be summarized as follows:

Main Request
Article 123(2) EPC

The application as filed related to enzymatic DNA molecules. These DNA molecules were exemplified by several different "families" which were initially generated from a starting pool of DNA by a process of "*in vitro* evolution" that identified DNA molecules that could self-cleave (Example 1, Figure 1). The members of

a family had differing sequences but all shared the ability to cis-cleave a fixed substrate sequence region specific to that family. Examples 2 and 4 disclosed two of the different families generated. Some of the cis-cleaving molecules could be converted into an intermolecular format (Examples 3 and 5). Example 5 identified two families of self-cleaving DNA molecules. These families cleaved the same target sequence but at different sites within that sequence. Various individuals from these families were cloned and sequenced (Tables 2 and 3) and several clones were tested in a self-cleavage reaction in which the RNA portion of the molecule was extended to be the sequence SEQ ID NO: 135. Figure 8 showed that the substrate for the trans-cleavage reaction with the "8-17" and "10-23" prototypes was identical to the extended RNA region used in the cis-cleaving reaction (SEQ ID NO: 135). The substrate binding arms of the two prototype enzymes "8-17" and "10-23" were reduced to 7 base pairs and the complementarity was improved (Figure 9) whilst maintaining the same RNA substrate (SEQ ID NO: 135).

Example 6 reported the preparation of improved enzymes based on the "8-17" and "10-23" motifs. For both enzymes, the sequence of the substrate could be changed without loss of catalytic activity so long as the substrate binding arms of the enzymes were changed in a complementary manner. Different combinations of RNA substrate and corresponding DNA enzyme in the substrate binding region revealed that the "10-23" motif could be generalized with respect to any substrate sequence and examples of RNA substrates different from the prototype substrate were prepared (Table 4) and shown to be cleaved by synthetic DNA enzymes containing the "10-23"

core flanked by substrate binding arms. A general formula setting out the sequence requirements for the "10-23" enzyme was shown in Formula II cited in original claim 1 of the application as filed.

The disclaimer introduced in claims 1 and 30 of the Main Request excluded all catalytic DNA molecules having the formula recited in original claim 1, i.e. the "10-23" structure, and having also the first and second binding regions that could bind through complementary base-pairing to a substrate nucleic acid of sequence SEQ ID NO: 135, i.e. the substrate molecule of the prototype enzymes "8-17" and "10-23" (Figures 8 and 9). Thus, the disclaimer of claims 1 and 30 of the Main Request excluded all "10-23" enzymes which bound to the prototype substrate and the remaining subject-matter was directed to "10-23" enzymes which did not bind to the prototype substrate.

This disclaimer did not generate new subject-matter. The skilled person directly and unambiguously derived from Example 6 that, once the random *in vitro* evolution process had allowed the identification of the "10-23" prototype enzyme and its prototype substrate (SEQ ID NO: 135), the sequence requirements of the enzyme were identified in order to enable substrates other than the prototype substrate to be cleaved. After the introduction of the disclaimer in claims 1 and 30, the subject-matter remaining in these claims corresponded to this class of "10-23" enzymes, i.e. those which cleaved substrates other than the prototype substrate. Thus, the skilled person was not presented with new information.

In summary, the disclaimer in claims 1 and 30 of the Main Request excluded catalytic molecules having the "10-23" structure and in which the first and second binding regions could bind through complementary base-pairing to a substrate nucleic acid of sequence SEQ ID NO: 135. As acknowledged in decision T 1068/07 (*supra*), the subject-matter excluded by this disclaimer was supported in the application as filed. The subject-matter remaining in claims 1 and 30 after the introduction of the disclaimer was directly and unambiguously disclosed in the application as filed. The exclusion of the disclaimed subject-matter did not modify the subject-matter remaining in claims 1 and 30 in such a way that the skilled person was presented with new technical information.

Thus, the claims of the Main Request fulfilled the requirements laid down in G 2/10 (*supra*) and consequently those of Article 123(2) EPC.

VIII. The appellant (applicant) requested that the decision under appeal be set aside and that a patent be granted on the basis of its Main Request or of Auxiliary Request I (filed on 25 May 2010 as Auxiliary Request II and III, respectively). Alternatively, it was requested that, should the board conclude that either the Main Request, or the Auxiliary Request I, meets the requirements of Article 123(2) EPC, the case be remitted to the examining division for examination of further substantive issues. As a precautionary measure, oral proceedings were requested, if the board intended to dismiss the appeal (Article 116 EPC).

Reasons for the Decision

Main Request
Article 123(2) EPC

1. According to the decision G 2/10 of the Enlarged Board of Appeal (*supra*), an amendment to a claim by a disclaimer disclaiming from it subject-matter disclosed in the application as filed infringes Article 123(2) EPC if the subject-matter remaining after the introduction of the disclaimer is not, be it explicitly or implicitly, directly and unambiguously disclosed to the skilled person using common general knowledge, in the application as filed (cf. point 1a of the Order of the decision G 2/10, *supra*; Section III *supra*).

2. In order for the board to decide whether the Main Request fulfils the requirements of Article 123(2) EPC, it is necessary to address the following questions: i) is the subject-matter of the disclaimer introduced in claim 1 of the Main Request - either explicitly or implicitly - directly and unambiguously disclosed in the application as filed?, and ii) is the subject-matter remaining in claim 1 of the Main Request after the introduction of the disclaimer in this claim - either explicitly or implicitly - directly and unambiguously derivable from the application as filed?.

3. The decision under appeal refers only to the disclaimer in claim 1. The disclaimer introduced now in claim 1 of the Main Request is also repeated in claim 30 of this Main Request. Claim 30 of the Main Request is directed to a method of engineering a catalytic DNA molecule that cleaves a preselected substrate nucleic acid

sequence in a target nucleic acid molecule and comprises the steps of: a) selecting a substrate of from 10 to 26 nucleotides in length in a target molecule; and b) synthesizing a DNA molecule as defined in claim 1 (cf. Section VI *supra*). Therefore, the conclusions reached below for claim 1 with regard to the introduction of the disclaimer also apply in all respects to the subject-matter of claim 30.

The disclosure of the application as filed and the subject-matter of the disclaimer introduced in claim 1

4. In its referral to the Enlarged Board of Appeal (cf. T 1068/07, Section II *supra*), this board, albeit in a different composition, acknowledged that the subject-matter of the disclaimer present now in claim 1 of the Main Request was disclosed in the application as filed. As a formal basis for this disclaimer, the board explicitly referred to page 85, lines 2 to 26 and Figure 9 of the application as filed, which report the results obtained in Example 5 of the application as filed (cf. point 14 of the Reasons in decision T 1068/07, *supra*).

5. As correctly stated by the appellant (cf. Section VII *supra*), the application as filed discloses an *in vitro* evolution process for generating, selecting and isolating - intramolecular (cf. Examples 1 and 2) and intermolecular (cf. Examples 3 and 4) - catalytic DNA molecules having site-specific endonuclease activity specific for a nucleotide sequence defining a cleavage site in a preselected substrate nucleic acid sequence, and having first and second binding regions flanking a core region, wherein said first and second substrate

regions have sequences capable of binding through complementary base-pairing to a first and a second portion, respectively, of said preselected substrate nucleic acid sequence.

6. In Example 5, the preselected (all-RNA) substrate nucleic acid sequence was a stretch of 12-highly conserved nucleotides (SEQ ID NO: 49) embedded within a longer DNA molecule that included a stretch of 50 random nucleotides (N_{50}) (SEQ ID NO: 50), which generated a population of putative enzymatic DNA molecules. After several rounds of *in vitro* evolution, enzymatic DNA molecules were selected for their ability to cleave a phosphoester within the embedded RNA target sequence. Several individual molecules from the population obtained after these rounds of *in vitro* selective amplification were cloned (cf. Tables 2 and 3) and their self-cleavage activity was measured. The self-cleavage reaction was easily converted to an intermolecular cleavage reaction by dividing the enzyme and substrate domains into separate molecules (cf. page 82, line 33 to page 83, line 1). Clone "10-23", which was identified as having a high level of activity, was chosen, together with clone "8-17", as a prototype molecule and characterized in detail, both structurally (nucleotide sequence, enzyme and substrate binding domains) and kinetically (cleavage site, turnover rates) (cf. Figure 8). The substrate binding arms of these molecules were further optimized by reducing them to 7 base-pairs on each side of the unpaired nucleotides demarcating the cleavage site (cf. Figure 9).

7. The catalytic core region of clone "10-23" shown in Figures 8 and 9 of the application as filed falls

within the more generic sequence SEQ ID NO: 122 referred to in original claim 1, which corresponds to the formula of the core region of claim 1 of the Main Request. The substrate nucleic acid sequence of clone "10-23", referred to on page 85 of the application as filed, is also shown in Figures 8 and 9 (intramolecular and intermolecular, respectively) and is identical to the sequence SEQ ID NO: 135 of the disclaimer present in claim 1 of the Main Request.

8. The board agrees with the appellant that the subject-matter of the disclaimer in claim 1 of the Main Request, namely the catalytic DNA molecules based on the "10-23" motif or prototype enzyme having a site-specific endonuclease activity specific for the substrate nucleotide sequence SEQ ID NO: 135, is explicitly disclosed in the application as filed.

The subject-matter remaining in claim 1 after introduction of the disclaimer

9. Example 6 of the application as filed is directed to the preparation of "universal substrate enzymes" based on the "8-17" and "10-23" motifs described in the previous examples of the application as filed. In Example 6, it is explicitly stated that "*(f)or both the 8-17 and 10-23 motif enzymes, the sequence of the substrate can be changed without loss of catalytic activity, so long as the substrate-binding arms of the enzyme were changed in a complementary manner*" (cf. page 87, lines 24 to 28). Further studies were carried out in Example 6 in order to define more precisely the sequence requirements of the catalytic core of these DNA molecules and, as a result of these studies, the

generic core region of the "10-23" motif was defined (cf. *inter alia* page 90, lines 26 to 29, page 96, lines 14 to 20, Figure 10 and original claim 1) and shown - by a survey of different combinations of RNA substrate and corresponding complementary DNA enzyme in the substrate binding region - to be generalizable with respect to **any** substrate sequence (cf. *inter alia* page 90, lines 29 to 33, page 92, line 23 to page 95, line 15, Table 4).

10. It is in fact this very specific subject-matter, namely catalytic DNA molecules having the "10-23" core region of the formula found in claim 1 of the Main Request having site-specific endonuclease activity specific for **any** (preselected) substrate nucleotide sequence **other than** the substrate nucleotide sequence SEQ ID NO: 135, which actually remains in claim 1 of the Main Request after the introduction of the disclaimer in this claim.

11. Thus, it follows from the above, that the criteria set out in point 1a of the Order of decision G 2/10 of the Enlarged Board of Appeal (cf. Section III and point 1 *supra*) are met by the disclaimer present in claim 1 of the Main Request and, accordingly, that this disclaimer fulfils the requirements of Article 123(2) EPC.

Further objections raised under Article 123(2) EPC

12. The appellant in the grounds of appeal protested against the inclusion of a section entitled "Final remarks not part of the present decision" in the appealed decision of the examining division, wherein the examining division raised several objections under Article 123(2) EPC (cf. point 5 on page 8 of

appellant's grounds of appeal filed on 12 June 2007 and point 5 on pages 6 and 7 of the decision under appeal). Nevertheless, the board referred to these objections in its communication pursuant to Article 15 RPBA (cf. point 11 on pages 5 and 6 of the board's communication; Section II *supra*).

13. Most of the remarks made, and objections raised, by the examining division related to the Sequence Listing containing SEQ ID NO:1 to SEQ ID NO:150 ("463.4.TXT SEQUENCE LISTING"), which was filed by the applicant with its letter of 15 January 2004 in order to include all nucleotide sequences that were referred to in the application as filed but not present in the original Sequence Listing containing SEQ ID NO:1 to SEQ ID NO: 101 (cf. pages 99 to 140 of the application as filed). In its letter, the applicant explicitly stated that "*To the best of my knowledge, the electronic form of the sequence listing corresponds to the printed form, and it does not include matter which goes beyond the content of the application as filed*". The board notes that the same objections were already raised - verbatim - by the examining division in its communication of 8 August 2006 annexed to the summons to oral proceedings and they were specifically addressed in detail by the applicant in its reply of 19 December 2006 in preparation for the oral proceedings before the examining division. Thus, none of the arguments put forward by the applicant has been discussed or even taken into account by the examining division in the Section "Final remarks not part of the present decision" of the decision under appeal.

14. The attention of the board has also been drawn to the "Decision of the President of the European Patent Office dated 28 April 2011 on the filing of sequence listings" and to the "Notice from the European patent Office dated 28 April 2011 on the filing of sequence listings" (OJ EPO, 6/2011, pages 372 and 376, respectively) which specify the requirements for the filing of sequence listings in respect of European patent applications and the subsequent filing of sequence listings and further refer to previous decisions of the President of the EPO and previous notices from the EPO that have since been superseded.

15. In view of the above considerations and taking into account that the objections raised by the examining division concerning the Sequence Listing result from amendments made in the original description, which presumably will have to be adapted again after a complete examination by the first instance, the board refrains from making any further comments with respect to this issue. Nevertheless, it is noted that the disclaimer in claims 1 and 30 of the Main Request explicitly refers to SEQ ID NO: 135 and that the subject-matter of dependent claims 14 and 40 of the Main Request refers to SEQ ID NO: 102 to 109. Should these references not be in accordance with the above cited "Decision of the President of the EPO" and "Notice from the EPO", these claims will have to be amended accordingly.

16. In the section "Final remarks not part of the present decision" of the decision of the examining division under appeal and in its letter of 8 August 2006 (cf. point 13 *supra*), the examining division raised an

objection under Article 123(2) EPC with regard to the term "R" in claim 1 and to the specification of the cleavage site as being 5' A-U 3' in claim 3. These objections were addressed by the applicant in its letter of 19 December 2006, which, as acknowledged by the board in its communication pursuant to Article 15(1) RPBA (cf. Section II *supra*), indicated a basis for these features. Basis for other amendments introduced into the claims were indicated by the applicant in its letter of 30 May 2005, in particular with references to the original claims, the description and the Figures of the application as filed.

17. No further objections under Article 123(2) EPC were raised by the examining division nor has the board a reason to raise any of its own at this stage of the appeal proceedings.

Conclusion

18. Thus, the subject-matter of claims 1 and 30 of the Main Request is considered to fulfil the requirements of Article 123(2) EPC.

Order

For these reasons it is decided that:

The decision under appeal is set aside and the case is remitted to the first instance for further prosecution on the basis of the Main Request (filed on 25 May 2010 as Auxiliary Request II).

The Registrar: The Chairman:

A. Wolinski M. Wieser