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Datasheet for the decision of 6 March 2007

Case Number: T 0192/06 - 3.3.08

Application Number: 94909526.9

Publication Number: 0682699

IPC: C12N 9/22

Language of the proceedings: EN

Title of invention:
Functional domains in Flavobacterium okeanokoites (FokI) restriction endonuclease

Patentee:
THE JOHNS-HOPKINS UNIVERSITY

Opponent:
CELLECTIS

Headword:
FokI endonuclease/JOHNS-HOPKINS

Relevant legal provisions:
EPC Art. 56

Keyword:
"Inventive step (no)"

Decisions cited:
T 0918/01, T 0278/03

Catchword:
-

Case Number: T 0192/06 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 6 March 2007

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Decision under appeal:
Decision of the Opposition Division of the European Patent Office posted 1 December 2005 revoking European patent No. 0682699 pursuant to Article 102(1) EPC.

Composition of the Board:

Chairman: L. Galligani
Members: P. Julià
C. Heath

Summary of Facts and Submissions

I. European patent no. 0 682 699 was granted on the basis of European patent application No. 94 909 526.9 (published as international application WO 94/18313) and was opposed on the grounds of Article 100(a) EPC. The patent was revoked by the opposition division for lack of inventive step (Article 56 EPC).

II. The patentee (appellant) lodged an appeal and filed the statements setting out the grounds of appeal. Three new documents were filed, among them Kim et al., 1996 (document A, Section IX *infra*).

III. The opponent (respondent) replied to the appellant's statements of grounds of appeal and filed five new documents.

IV. With the summons to the oral proceedings, the board sent a communication to the parties under Article 11(1) of the Rules of Procedure of the Boards of Appeal (RPBA). The parties were informed therein of the board's preliminary opinion on the relevant issues.

V. With letter of 6 February 2007, the appellant replied to the communication of the board and filed the main request (claims as granted) and auxiliary requests 1 to 10. Three expert declarations and three further documents were filed, among them Kim et al., 1994 (document B, Section IX *infra*).

VI. With letter of 2 March 2007, the respondent replied to the communication of the board, thereby filing three additional post-published documents.

VII. Oral proceedings took place on 6 March 2007. At the end of these oral proceedings, the appellant withdrew all auxiliary requests on file, including two auxiliary requests filed during the oral proceedings (auxiliary requests 4a and 10a), and maintained the main request as its sole request.

VIII. The claims of the sole **request** before the board were those as granted and corresponded to those underlying the decision under appeal as well. Claim 1 read as follows:

"1. A DNA construct comprising:

(i) a first DNA segment encoding the catalytic domain of a Type IIS endonuclease which contains the cleavage activity of said Type IIS endonuclease;

(ii) a second DNA segment encoding a sequence-specific recognition domain other than the recognition domain of said Type IIS endonuclease;

(iii) a third DNA segment comprising one or more codons, wherein said third DNA segment is inserted between said first DNA segment and said second DNA segment; and

(iv) a vector

wherein said first DNA segment, said second DNA segment and said third DNA segment are operably linked to said vector so that a single protein is produced."

Claims 2 to 7 related to further embodiments of claim 1. Claims 8 to 10 were directed to prokaryotic cells

comprising the DNA construct of claim 1. Claim 11 related to an isolated hybrid Type IIS endonuclease produced by the prokaryotic cell of claim 8.

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

D1: L. Li et al., Proc. Natl. Acad. Sci. USA, May 1992, Vol. 89, pages 4275 to 4279;

D2: EP-B-0 318 554 (publication date of the application: 7 June 1989);

A: Y-G. Kim et al., Proc. Natl. Acad. Sci. USA, February 1996, Vol. 93, pages 1156 to 1160;

B: Y-G. Kim et al., J. Biol. Chem., 16 December 1994, Vol. 269(50), pages 31978 to 31982.

X. The appellant's arguments may be summarized as follows:

Article 56 EPC

Document D1, the closest prior art, disclosed two separate functional domains in the Type IIS restriction endonuclease *Fok*I. Digestion of the *Fok*I enzyme by trypsin resulted in a 41 kDa amino terminal fragment and a 25 kDa carboxy terminal fragment which comprised, respectively, the *Fok*I sequence-specific recognition domain (DNA binding domain) and the *Fok*I cleavage (catalytic) domain. The isolated *Fok*I catalytic domain cleaved, non-specifically, both methylated and non-methylated DNA substrates in presence of $MgCl_2$. In order for site-specific cleavage to occur, a cleavage

signal had to be transmitted from the DNA binding domain to the catalytic domain, probably through allosteric interactions. The presence of these interactions was confirmed by further experimental evidence, in particular by the different patterns of trypsin digestion of the *Fok*I enzyme obtained with and without a DNA substrate. Document D1 further speculated that it could be feasible to construct hybrid endonucleases of different sequence specificity by linking other DNA binding domains to the *Fok*I cleavage domain. Although the level of knowledge of the authors of document D1 was clearly higher than the one of the average person skilled in the art, they were by no means sure that a functional hybrid endonuclease could be constructed. Moreover, no guidance was provided of how the "linking" of other DNA binding domains to the *Fok*I cleavage domain could be effected, particularly in a way that the allosteric regulation of the specific cleavage activity of the wild-type *Fok*I was retained.

The patent in suit went far beyond what was disclosed in document D1 and described the insertion of linkers between the catalytic domain and the sequence-specific recognition domain of the Type IIS endonuclease *Fok*I. The resulting recombinant *Fok*I endonuclease was functionally active and cleaved at positions shifted from the recognition site of the wild-type *Fok*I enzyme. Thus, the patent in suit provided further technical support for the construction of the claimed hybrid Type IIS restriction endonucleases.

Starting from this prior art, the technical problem to be solved was the provision of a functional hybrid Type IIS endonuclease with different specificity. Whereas

the term "functional" required sequence-specific DNA recognition in combination with the cleavage activity of a Type IIS endonuclease (a regulated site-specific DNA cleavage), the term "different specificity" required cleavage at a site different from the cleavage site of the wild-type enzyme.

The patent in suit showed that the insertion of four or seven codons between the sequence-specific recognition and the cleavage domains of the *Fok*I endonuclease destabilized this enzyme, which however remained functionally active. Similar studies were disclosed in the post-published document B, which reported the construction of several insertions between the DNA binding domain and the catalytic domain of the *Fok*I endonuclease. All tested *Fok*I insertion mutants retained their function and it was concluded that large insertions did not disrupt the activity of the enzyme. Further post-published evidence demonstrated that hybrid Type IIS endonucleases with linkers of different length and nature (with or without secondary structure) were also capable of sequence-specific cleavage of substrate DNA. There was no evidence on file showing the contrary. Thus, the technical problem was solved for the whole range of the subject-matter covered by the claims.

This solution was not rendered obvious by document D1 alone or in combination with other prior art. Document D1 referred to the presence of allosteric interactions between the two domains of the *Fok*I endonuclease and showed that trypsin digestion of this enzyme gave different results in presence and in absence of a DNA substrate. Thus, the binding to a DNA substrate caused

major structural alterations showing that regulated site-specific cleavage of DNA substrates indeed required a functional interaction between the DNA binding domain and the cleavage domain. The skilled person would have deduced therefrom that site-specific cleavage at a predetermined position from the recognition site could only have occurred if a signal was successfully transmitted from the DNA binding domain to the catalytic domain. This deduction was further supported by the observation that the isolated *Fok*I catalytic domain had completely lost its specificity. Thus, the DNA binding domain not only was capable of suppressing the non-specifically cleavage activity of the *Fok*I catalytic domain in presence of a DNA substrate (with recognition sites) but it was also capable of blocking the cleavage activity of the *Fok*I catalytic domain in presence of a non-substrate DNA (without recognition sites). The effect of inserting a peptide linker (for which no guidance was given in document D1) and a heterologous DNA binding domain on these allosteric and suppressing interactions was not obvious at all. In fact, the skilled person would have understood that massive obstacles were to be overcome since those structural alterations could negatively affect any allosteric signal transmission between the sequence-specific recognition domain and the cleavage domain as well as the structural interrelationship between these domains. In the absence of this information, the reference to hybrid endonuclease in document D1 was nothing more than a mere speculation. Although the skilled person could have followed this speculation, massive obstacles had to be faced and, without any technical guidance, there was no actual motivation to follow it.

This technical guidance or information could not have been derived from other prior art, which referred to fusion proteins substantially different from the claimed hybrid Type IIS endonucleases. In particular, document D2 disclosed fusion proteins of an antigen-binding site domain and an effector domain connected by a peptide linker which preserved the functions of the individual domains and, contrary to the patent in suit, avoided any allosteric interaction between the two domains. Thus, by using a peptide linker for avoiding allosteric interactions, document D2 even taught away from the solution proposed by the patent in suit.

In the light of this prior art and of the disclosure of document D1, there was no reason to expect that the presence of a heterologous DNA binding domain and a peptide linker would modify the unregulated and non-specific DNA cleavage activity of the isolated *Fok*I catalytic domain. It could not be expected that a hybrid Type IIS endonuclease would be functional and exhibit a sequence-specific and a cleavage activity different from the wild-type enzyme. This was supported by the expert declarations on file that reflected the opinion of the scientific community at the priority date of the patent in suit. These declarations also showed that the skilled person would have expected to encounter real technical difficulties when preparing a functional hybrid type IIS endonuclease. A hope to succeed was not be confused with a reasonable expectation of success.

XI. The respondent's arguments may be summarized as follows:

Article 56 EPC

Document D1, the closest prior, identified two distinct functional domains of the *Fok*I endonuclease, namely a 41 kDa DNA binding domain and a 25 kDa cleavage domain (positions 1-382 and 383-578, respectively, of the *Fok*I sequence, as derived from their N-terminal sequence disclosed in Table 1). The construction of hybrid endonucleases by linking the catalytic domain of the *Fok*I endonuclease with a heterologous binding domain was explicitly suggested in this document and several known binding domains were explicitly indicated therein. In the context of document D1, the term "linking" was clearly understood as providing a peptide linker (a DNA coding therefor) between the two identified domains.

Starting from this prior art, the objective problem to be solved was the provision of alternative functional hybrid Type IIS endonucleases. The solution proposed by the patent in suit, namely the introduction of a linker between a catalytic domain of a Type IIS endonuclease and a heterologous sequence-specific recognition domain, was obvious to the skilled person.

Document D1 already provided all the information required for solving this technical problem, namely the identification of the distinct domains of the *Fok*I endonuclease and the demonstration that the isolated domains were functionally active. The construction of hybrid endonucleases by linking a heterologous sequence-specific recognition domain to the Type IIS endonuclease catalytic domain was explicitly suggested

by this document and the term "linking" was clearly understood by the skilled person. Different linker peptides and criteria for selecting them were well-known in the prior art as shown *inter alia* in document D2. Moreover, document D1 itself provided sufficient information for selecting a suitable linker, in particular, the length (maintain possible allosteric interactions) and structure (retain the original binding to DNA substrate at one face of the DNA helix and the cleavage at another point on the next helical turn). No inventive contribution could be seen in the selection of the length and/or nature of the linker. Furthermore, since none of these features was defined in the claims, none of them could be considered as an essential feature for solving the technical problem. On the other hand, there was no evidence on file showing that all possible (long, arbitrary) linkers actually solved this problem. Therefore, no inventive skill was required to follow the indications of document D1 and achieve thereby the claimed hybrid endonucleases.

Although document D1 referred to the presence of possible allosteric interactions between the two domains of the *Fok*I endonuclease and showed the non-specific cleavage activity of the isolated *Fok*I catalytic domain, the skilled person was not dissuaded from following the suggestion made in this document and thereby construct the proposed hybrid endonucleases. In fact, the allosteric interactions were referred to at the beginning of document D1 only as a possible mechanism of action of the *Fok*I endonucleases. However, a cleavage-specificity linked solely to the binding of the sequence-specific recognition domain to the DNA substrate (DNA binding specificity) was also indicated

as another possible model at the end of document D1. Document D1 explicitly indicated other known sequence-specific recognition domains with no structural similarity to the *Fok*I DNA binding domain for the construction of the hybrid endonucleases. There was no reason for the skilled person to expect the first model to be more valid or true than the second one. This was all the more so, since there was no evidence supporting the first model and the document provided a clear suggestion for the skilled person to follow based on the second one. In this case, the skilled person would have expected a heterologous DNA binding domain linked to the *Fok*I catalytic domain to redirect the non-specific cleavage activity of the isolated *Fok*I catalytic domain to a sequence-specific cleavage. Since all elements and the required information were known, there was no reason for the skilled person not to follow the explicit suggestion made in document D1. This was in fact what was exactly done in the post-published documents on file, which further demonstrated that no technical difficulties were actually encountered when following this suggestion.

If, starting from document D1 as the closest prior art, the technical problem to be solved was defined as the provision of alternative functional hybrid Type IIS endonuclease having a different cleavage specificity than the wild-type *Fok*I endonuclease (cleavage at 9/13 nucleotides away from the sequence-specific recognition site), then the claimed subject-matter did not solve this technical problem. From the patent in suit it could be derived that the insertion site of the linker as well as the length and nature of the linker were

essential features for solving the problem. The absence of these features in the claims implied that not all claimed subject-matter solved the technical problem.

XII. The appellant (patentee) requested that the decision under appeal be set aside and that the patent be maintained as granted, all other pending requests having been withdrawn.

XIII. The respondent (opponent) requested that the appeal be dismissed.

Reasons for the Decision

1. In the present appeal proceedings, the sole issue before the board concerns inventive step.

The closest prior art

2. In accordance with the case law of the Boards of Appeal which defines the closest prior art as a document disclosing subject-matter conceived for the same purpose or aiming at the same objective as the claimed invention and having the most relevant technical features in common (cf. "Case Law of the Boards of Appeal of the EPO", 4th edition 2001, I.D.3.1, page 102), the board concurs with the parties that document D1 represents the closest prior art.

3. Document D1 identifies by trypsin digestion of the *Fok*I endonuclease two distinct fragments that comprise the functional domains of this enzyme, namely a 41 kDa fragment which binds to DNA in a sequence-specific

manner (sequence-specific recognition domain or DNA binding domain) and a 25 kDa fragment which cleaves a DNA substrate (cleavage or catalytic domain). The amino-terminal sequence of these fragments is disclosed in Table 1 (cf. page 4278, left-hand column) and these isolated fragments are shown to be functionally active. In particular, the purified *Fok*I catalytic domain "*cleaves nonspecifically both unmethylated DNA substrate ... and methylated DNA substrate ... in the presence of* $MgCl_2$" and, since the resulting products are small, the catalytic domain is "*relatively non-specific in cleavage*" (cf. sentence bridging pages 4277 and 4278 and page 4278, left-hand column, first full sentence). Document D1 refers to further mutational analysis required for precisely defining the domain structure of the *Fok*I enzyme and explicitly states that "*the molecular structure of the enzyme suggests that it may be feasible to construct chimeric endonucleases of different sequence specificity by linking other DNA-binding proteins (e.g., zinc finger motifs, homeo domain motifs, and DNA-binding domains of lambda, lac repressors, cro, etc.) to the cleavage domain of FokI endonuclease*" (cf. page 4279, right-hand column).

4. The patent in suit does not disclose any hybrid *Fok*I endonuclease but it shows that the insertion of a linker of four (or seven) codons "*near the trypsin cleavage site of FokI that separates the recognition and cleavage domains*" (cf. paragraph [0077] of the patent, Example XI) does not alter the DNA sequence specificity or DNA recognition mechanism of the enzyme (cf. paragraph [0080], Example XIII) and that for the resulting *Fok*I enzyme "*the cut sites are shifted from the recognition site on both strands of the*

substrate ... as compared to the wild-type enzyme" (cf. paragraph [0081], Example XIV). These results support the feasibility of hybrid *Fok*I endonucleases, i.e. the subject-matter actually claimed.

<u>The objective technical problem to be solved</u>

5. Starting from this closest prior art, the objective technical problem to be solved is seen in the provision of functional chimeric Type IIS endonucleases of different sequence specificity, in particular hybrid *Fok*I endonucleases.

6. It is worth noting at this point that the claimed subject-matter does not require any specific level of endonuclease activity nor is any particular one referred to in the patent in suit. Likewise, there is no requirement for a particular cleavage-specificity (predetermined distance away the from sequence-specific recognition site) or for a yield or efficiency of this cleavage-specificity. The patent in suit refers only to "*multiple cut sites*" with "*major cut sites*" and "*a small amount of cleavage similar to the wild-type enzyme*" (cf. paragraphs [0082] and [0083]). Thus, in accordance with the established case law of the Boards of Appeal, these features cannot be taken into consideration in determining the problem underlying the invention (cf. "Case Law", *supra*, I.D.4.4, page 108).

<u>Is the problem solved over the whole range claimed?</u>

7. Although the production of a hybrid Type IIS endonuclease is not actually exemplified in the patent in suit, there is post-published evidence on file that

shows the production of these hybrid Type IIS endonucleases and in particular of hybrid *Fok*I endonucleases. Document A (cited as expert opinion) discloses a hybrid restriction enzyme comprising a Zinc finger DNA binding domain and a *Fok*I cleavage domain linked by a glycine $(Gly_4Ser)_3$ linker (cf. page 1157, Figure 1). The rate and efficiency of cleavage of the hybrid endonuclease are much lower compared to the wild-type *Fok*I enzyme but the reaction proceeds almost to completion (>95% cleavage) within 4 hr (15 min for the wild-type enzyme) (cf. page 1157, right-hand column, line 12 from the bottom). In the post-published document B (cited as expert opinion) a similar linker and linkers of other length (from 4 to 23 residues) and nature are inserted between the two domains of the wild-type *Fok*I enzyme and endonuclease activity is reported for all of them (cf. page 31980, Table 1). Although "*larger insertion mutants show partial digests*", "*these reactions proceed to completion either by increasing the enzyme concentration or by digesting for longer time periods*" (cf. page 31979, right-hand column, line 4 from the bottom). Thus, the post-published evidence on file shows that, ruling out interpretations of the term "linker" that do not make technical sense (cf. "Case Law", *supra*, II.B.4.1, page 168), the length and nature of the linker are not essential for solving the technical problem which is thus solved over the whole scope of the claims.

<u>Is the proposed solution obvious?</u>

8. It has been argued by the appellant that since there is no guidance in document D1 as to how to link the sequence-specific recognition domain and the *Fok*I

cleavage domain, then this "linking" is not obvious to the skilled person (cf. Section X *supra*). Document D1 refers to the cloning of the *Fok*I endonuclease and to the construction of expression constructs (cf. page 4275, right-hand column) and thus, lies in the field of genetic engineering. There is ample prior art on file showing that the term "linker" would be immediately understood by the skilled person working in this field as a peptide linker (a DNA coding therefor). Document D2, which has been referred to as one representative of such knowledge in the art, exemplifies, although in a different context, routine considerations of the skilled person when designing those linkers for a certain purpose, in particular relating to the linker length and nature (propensity for secondary structure) (cf. page 10, lines 14 to 21 and 40 to 42, page 17, lines 20 to 32). This prior art rules out interpretations of the term "linking" that, in the context and the technical field of document D1, do not make technical sense. Thus, the board considers that the "linking" - explicitly referred to in document D1 - is obvious to the skilled person and that no inventive skill can be seen in performing it in a classical manner. This is all the more so, since neither the length nor the nature of the linker are essential features for solving the technical problem (cf. point 7 *supra*).

A reasonable expectation of success?

9. According the established case law of the Boards of Appeal, in cases where a course of action could be considered obvious within the meaning of Article 56 EPC, it still has to be assessed if the skilled person would

have carried it out in the expectation of success, i.e. obviousness is not only at hand when the results are clearly predictable but also when there is a reasonable expectation of success (cf. "Case Law", *supra*, I.D.6.2, page 117). In the present case, it has been argued that the references to allosteric interactions and the non-specific cleavage activity of the isolated *Fok*I catalytic domain shown in document D1 would have dissuaded the skilled person from following the suggestion to construct hybrid *Fok*I endonuclease made also in this document (cf. Section X *supra*).

10. Whereas the cleavage activity of the isolated *Fok*I catalytic domain could not be anticipated without experimental evidence, the presence of a non-specific cleavage activity is not at all surprising since Type IIS endonucleases are characterized by having a sequence-specificity defined by the DNA binding site and **not** by the cleavage site. If the isolated *Fok*I catalytic domain retains the non-specific cleavage activity when relieved from possible steric constrains and structural interactions with the sequence-specific recognition domain of the *Fok*I endonuclease, it is also reasonable to expect that the presence of a peptide linker and a heterologous DNA binding domain will (re)introduce certain steric constrains and structural interactions to the isolated *Fok*I cleavage domain and thereby redirect the non-specific cleavage activity of this *Fok*I domain. All the more so, since the sequence-specific recognizing domains referred to in document D1 are known to bind in a sequence-specific manner when being part of other hybrid proteins. Therefore, it is reasonable to expect that the sequence-specific binding is also present when linked

with the isolated *Fok*I cleavage domain. In view of the different structure of the *Fok*I DNA binding domain and the heterologous DNA binding domains suggested in document D1, it is also reasonable to expect that the cleavage specificity of the hybrid *Fok*I endonuclease will be altered in comparison to the one of the wild-type *Fok*I endonuclease.

11. In fact, the expectation of success usually depends on the technical problem to be solved. If for very ambitious problems, important difficulties might *a priori* be expected, less ambitious problems might normally be associated with higher expectation of success. In the present case, the technical problem to be solved is not particularly ambitious, since there is no requirement for a specific level of activity for the claimed hybrid Type IIS endonucleases nor a particular cleavage-specificity or efficiency thereof (cf. point 6 *supra*). Whereas a reasonable expectation of success should not be confused with the understandable "hope to succeed" (cf. "Case Law", *supra*, I.D.6.2, page 117), it is also true that absolute certainty is not required and is not a criterion for assessing the expectation of success (cf. T 918/01 of 6 October 2004 and T 278/03 of 18 January 2005, points 9.1. and 13, respectively, of the Reasons for the Decision).

12. In the present case, although the skilled person could not be completely certain that the suggested hybrid Type IIS endonucleases would be functionally active, the board considers that, for the reasons given above, a reasonable expectation was given. There is also (post-published) evidence on file showing that the skilled person would have encountered no real technical

difficulties when following the explicit suggestion made in document D1 (cf. "Case Law", *supra*, I.D.6.2, page 119).

Conclusion

13. It follows from all above that the claimed subject-matter does not fulfil the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

D. Magliano L. Galligani