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(54) Title: METHODS AND COMPOSITIONS FOR CONTROLLING EFFICACY OF RNA SILENCING

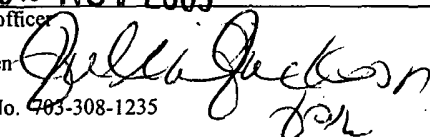
(57) Abstract: Based at least in part on an understanding of the mechanisms by which small RNAs (e.g., naturally-occurring miRNAs) mediate RNA silencing in plants, rules have been established for determining, for example, the degree of complementarity required between an RNAi-mediating agent and its target, i.e., whether mismatches are tolerated, the number of mismatches tolerated, the effect of the position of the mismatches, etc. Such rules are useful, in particular, in the design of improved RNAi-mediating agents which allow for more exact control of the efficacy of RNA silencing.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/17130

A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : C07H 21/00 US CL : 536/23.5 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 536/23.5 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Continuation Sheet		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ELBASHIR et al. Functional anatomy of siRNAs for mediating efficient RNAi in Drosophila melanogaster embryo lysate. The EMBO Journal. 2001, Vol. 23, pp. 6877-6888.	1-3 and 5-10
E, X	US 2004/0137471 (VICKERS et al.) 15 July 2004 (15.07.04) (especially pg. 7,[0060] and pg. 23, example 14, table VIII)	1, 5-7 and 10-11
X	WO 01/75164 A2 (TUSCHL et al.) 11 October 2001 (11.10.01)(see especially pg. 45, line 15 through pg. 46, line 25)	1, 5-10
☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
* Special categories of cited documents:		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed	
Date of the actual completion of the international search 05 August 2005 (05.08.2005)		Date of mailing of the international search report 02 NOV 2005
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230		Authorized officer Jon B. Ashen  Telephone No. 403-308-1235

INTERNATIONAL SEARCH REPORT

International application No.

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-11

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

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BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group 1, claim(s) 1-11, drawn to a method of making an RNAi agent comprising substituting at least one terminal nucleotide of the RNAi agent with a nucleotide which does not form a Watson-Crick base pair with the corresponding nucleotide in the target mRNA.

Group 2, claim(s) 12-24 and 27-38, drawn to an RNAi agent comprising at least one terminal nucleotide of the RNAi agent with a nucleotide which does not form a Watson-Crick base pair with the corresponding nucleotide in the target mRNA and a vector, a cell and a composition comprising said RNAi agent.

Group 3, claim(s) 25-26, drawn to a method of silencing a target mRNA in a cell or subject comprising contacting said cell or subject with an RNAi agent of any one of claims 1-24.

Group 4, claim(s) 39, drawn to an isolated *Arabidopsis thaliana* dicer-like enzyme.

The inventions listed as Groups 1-4 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

There is no special technical feature linking the inventions set forth in groups 1-4.

The special technical feature of Group 1 (as set forth in claim 1) is a method of making an RNAi agent comprising substituting at least one terminal nucleotide of the RNAi agent with a nucleotide which does not form a Watson-Crick base pair with the corresponding nucleotide in the target mRNA.

However, Elbashir et al. (The EMBO Journal, December 2001, Vol. 20(23): pp. 6877-6888) disclose a method of making an RNAi agent comprising substituting the 2 nucleobases that comprised the 2 nucleobase 3' overhangs of their disclosed siRNAs with various combinations of nucleotides, including terminal nucleotides, that do not form base pairs with the corresponding nucleotides of the target mRNA (see in particular page 6884, sections "Sequence effects and 2'-deoxy substitutions in the 3' overhang" and "Sequence specificity of target recognition").

Therefore, the special technical feature of group 1 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of Group 1 is a method of making an RNAi agent comprising substituting at least one terminal nucleotide of the RNAi agent with a nucleotide which does not form a Watson-Crick base pair with the corresponding nucleotide in the target mRNA.

The special technical feature of group 2 is an RNAi agent comprising at least one terminal nucleotide of the RNAi agent with a nucleotide which does not form a Watson-Crick base pair with the corresponding nucleotide in the target mRNA and a vector, a cell and a composition comprising said RNAi agent.

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The special technical feature of group 3 is a method of silencing a target mRNA in a cell or subject comprising contacting said cell or subject with an RNAi agent of any one of claims 1-24.

The special technical feature of group 4 is an isolated *Arabidopsis thaliana* dicer-like enzyme.

Continuation of B. FIELDS SEARCHED Item 3:

EAST, STN (medline, biosis, embase, caplus): search terms: sirna, rna, mismatch, G:U, substitution, wobble, terminal, modification, a-g, c-u