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Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

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06 December 2018 (06.12.2018)

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(54) Title: POLYPEPTIDES WITH TYPE V CRISPR ACTIVITY AND USES THEREOF

(57) Abstract: Disclosed herein are novel polypeptides having nuclease activity. The Mmc3 polypeptides function as Class 2 Type V effectors, and catalyze double stranded breaks in nucleic acid strands. The polypeptides are useful, for example, for gene editing systems such as CRISPR, to make site specific alterations of target nucleic acid sequences.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/27650

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/46, A61K 48/00, C07K 19/00 (2018.01)
CPC - C12N 9/22, A61K 38/465, A61K 48/0058

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)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2016/0208243 A1 (THE BROAD INSTITUTE, INC) 21 July 2016 (21.07.2016); Abstract, para [0010], [0033], [0035], [0447]	45, 47
A		1-22, 46, 48
A	Uniprot Submission A0A0C2W1L1, Online database, Uncharacterized protein, 1 April 2015 [online]. [Retrieved on 15 October 2018]. Retrieved from the internet <URL: https://www.uniprot.org/uniprot/A0A0C2W1L1> Entire document	1-22, 45-48
A	WO 2016/183531 A1 (SYNLOGIC, INC) 17 November 2016 (17.11.2016); para [0230]	1

☐ 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
"E" earlier application or patent but published on or after the international filing date
"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)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"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
"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
"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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 October 2018	31 OCT 2018

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/27650

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:
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 I+, claims 1-24, 45-48, directed to an engineered, non-naturally occurring Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system comprising an Mmc3 effector polypeptide, or a nucleotide sequence encoding an Mmc3 effector polypeptide. The CRISPR-Cas system will be searched to the extent that the effector polypeptide encompasses the sequence of SEQ ID NO: 1. It is believed that claims 1-22 and 45-48 encompass this first named invention, and thus these claims will be searched without fee to the extent that the effector polypeptide encompasses SEQ ID NO: 1. Additional effector polypeptide sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected effector polypeptide sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be the sequence of SEQ ID NO: 2 (claims 1-22 and 45-47). *****Continued in Supplemental Box*****

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 additional fees, this Authority did not invite payment of additional fees.

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.:
1-22 and 45-48 limited to SEQ ID NO: 1

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/27650

Continuation of Box NO. III (Observations where unity of invention is lacking):

Group II+, claims 25-44, directed to a method of modifying one or more target nucleic acid sequences in vivo with a CRISPR-Cas system. Group II+ will be searched upon payment of additional fees. The method may be searched, for example, to encompass the sequence of SEQ ID NO: 1 for an additional fee and election as such. It is believed that claims 25, 27-44 read on this exemplary invention. Additional effector polypeptide sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected effector polypeptide sequence(s). Failure to clearly identify how any paid additional invention fees are to be applied to the"+" group(s) will result in only the first claimed invention to be searched. Another exemplary election would be the sequence of SEQ ID NO: 2 (claims 25, 27-44).

Note, Mmc3 ORF3 polypeptides (claims 23, 24 and 26) are not part of the first or second election of Groups I+ and II+, as they correspond to SEQ ID Nos: 50-58 (see applicant specification para [0025]).

Group III, claims 49-53, directed to an engineered, non-naturally occurring CRISPR-Cas system comprising one or more nucleic acid constructs comprising a Cpfl effector polypeptide.

The inventions listed as Groups I+, II+ and III do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

Group I+ has the special technical feature of an Mmc3 effector polypeptide, that is not required by Group II+ or III.

Group II+ has the special technical feature of modifying one or more target nucleic acid sequences in vivo, comprising delivering to a cell comprising one or more nucleic acid molecules, that is not required by Group I+ or III.

Group III has the special technical feature of a Cpfl effector polypeptide, that is not required by Group I+ or II+.

The inventions of Groups I+ and II+ each include the special technical feature of a unique amino acid sequence. Each amino acid sequence encodes a unique peptide, and is considered a distinct technical feature.

Shared technical features

The inventions of Groups I+, II+ and III share the common technical feature of an engineered, non-naturally occurring Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system comprising: an Mmc3 or Cpfl effector polypeptide, or one or more nucleotide sequences encoding a effector polypeptide; and one or more engineered guide RNAs comprising a guide sequence, wherein the one or more guide RNAs is designed to form a complex with the effector polypeptide and wherein the one or more guide RNAs comprises a guide sequence designed to hybridize with one or more target nucleic acid molecules, wherein the guide RNA and the effector polypeptide do not naturally occur together.

No technical features are shared between the effector polypeptide sequences of Groups I+ and II+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ and II+ were considered to further share the technical features of including: wherein the effector polypeptide is an Mmc3 or Cpfl effector polypeptide, a nucleic acid molecule encoding a nuclease-deficient mutant of an Mmc3 effector polypeptide, and a method of modifying one or more target nucleic acid sequences in vivo, comprising delivering to a cell a composition comprising the CRISPR-Cas system, these shared technical features are made obvious by US 2016/0208243 A1 to The Broad Institute, Inc. et al., (hereinafter 'Broad')

Broad teaches an engineered, non-naturally occurring Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system comprising: an effector polypeptide, or one or more nucleotide sequences encoding an effector polypeptide (para [0010] -The invention provides a method of modifying sequences associated with or at a target locus of interest, the method comprising delivering to said locus a non-naturally occurring or engineered composition comprising a putative Type V CRISPR-Cas loci effector protein and one or more nucleic acid component), and one or more engineered guide RNAs comprising a guide sequence designed to hybridize with one or more target nucleic acid molecules and designed to form a complex with the effector polypeptide (Abstract- comprising a novel DNA or RNA-targeting CRISPR effector protein and at least one targeting nucleic acid component like a guide RNA.; [0012] the Cpf1 effector protein forms a complex with one nucleic acid component...induction of modification of sequences associated with or at the target locus of interest can be Cpf1 effector protein-nucleic acid guided. In a preferred embodiment the one nucleic acid component is a CRISPR RNA (crRNA)...the one nucleic acid component is a mature crRNA or guide RNA...the seed sequence is critical for recognition and/or hybridization to the sequence at the target locus), wherein the guide RNA and the effector polypeptide do not naturally occur together (para [0447] wherein the Cas protein and the guide RNA do not naturally occur together), however fails to teach wherein the effector polypeptide is Mmc3.

*****Continued in Supplemental Box*****

Continuation of Previous Page:

However, Broad does teach Class 2, Type V RNA-guided nucleases, or effector polypeptides, that include an RuvC domain characterized by three catalytic motifs with spacing, and effector peptide sequences with homology to "Mmc3" polypeptides (Abstract- In particular, the invention provides non-naturally occurring or engineered DNA or RNA-targeting systems comprising a novel DNA or RNA-targeting CRISPR effector protein and at least one targeting nucleic acid component like a guide RNA.; para [0010] -The invention provides a method of modifying sequences associated with or at a target locus of interest, the method comprising delivering to said locus a non-naturally occurring or engineered composition comprising a putative Type V CRISPR-Cas loci effector protein and one or more nucleic acid components, wherein the effector protein forms a complex with the one or more nucleic acid components and upon binding of the said complex to the locus of interest the effector protein induces the modification of the sequences associated with or at the target locus of interest.; [0033] -The invention also encompasses computational methods and algorithms to predict new Class 2 CRISPR-Cas systems and identify the components therein.; [0035] - the one or more mutations or the two or more mutations to be in a catalytically active domain of the effector protein comprising a RuvC domain.; [0035] -.a vector encodes a nucleic acid-targeting effector protein that may be mutated with respect to a corresponding wild-type enzyme such that the mutated nucleic acid-targeting effector protein lacks the ability to cleave one or both DNA or RNA strands of a target polynucleotide containing a target sequence. As a further example, two or more catalytic domains of a Cas protein (e.g. RuvC I, RuvC II, and RuvC III or the HNH domain of a Cas9 protein) may be mutated to produce a mutated Cas protein substantially lacking all DNA cleavage activity.; SEQ ID NO: 100 has 6.3% homology). In light of the fact that Broad teaches an effector sequence with the claimed parameters describing Mmc3 in the applicants' specification (instant specification - para [0010] "Part of a CRISPR system...Mmc3 effector polypeptides comprise a family of Class 2, Type V traguide RNA independent RNA-guided nucleases, or effector polypeptides, as disclosed in detail herein. The Mmc3 family forms a distinct group of RNA-guided endonucleases that include an RuvC domain characterized by three catalytic motifs with characteristic spacing"), it would have been obvious to an artisan of ordinary skill in the art to design a "Mmc3" effector polypeptide with the methods taught by Broad, in order to optimize effector function for a given CRISPR-Cas system.

Broad further teaches a nucleic acid molecule encoding a nuclease-deficient mutant of the effector polypeptide (para [0322] -Nuclease deficient Cas (e.g. Cpf1) proteins are useful for RNA-guided target sequence dependent delivery of functional domains. The invention provides methods and mutations for modulating binding of Cas (e.g. Cpf1) proteins.).

Broad further teaches a method of modifying one or more target nucleic acid sequences in vivo, comprising delivering to a cell a composition comprising the CRISPR-Cas system (para [0010] -The invention provides a method of modifying sequences associated with or at a target locus of interest, the method comprising delivering to said locus a non-naturally occurring or engineered composition comprising a putative Type V CRISPR-Cas loci effector protein and one or more nucleic acid components, wherein the effector protein forms a complex with the one or more nucleic acid components and upon binding of the said complex to the locus of interest the effector protein induces the modification of the sequences associated with or at the target locus of interest).

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+, II+ and III inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

Note, Claim 50 is objected to for lack of antecedent basis. As drafted, claim 50 depends from claim 1, but claim 1 does not recite a "Cpfl effector". For the purposes of this ISA Search and Written Opinion, claim 50 is construed as though depending from claim 49.