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(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF UTAH RESEARCH FOUNDATION** [US/US]; 615 Arapeen Drive, Suite 110, Salt Lake City, UT 84108 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PRESCOTT, Stephen** [US/US]; Huntsman Cancer Institute, 2000 Circle of Hope, Rm 5360, Salt Lake City, UT 84108 (US). **TOPHAM, Matthew** [US/US]; Huntsman Cancer Institute, 2000 Circle of Hope, Room 5363, Salt Lake City, UT 84108 (US). **BAZAN, Nicolas** [US/US]; Neuroscience Center, LSU Health Sciences Center, 2020 Gravier Street, Suite D, New Orleans, LA 70112 (US). **RODRIGUEZ DE TURCO, Elena** [US/US]; Neuroscience Center, LSU Health Sciences Center, 2020 Gravier Street, Suite D, New Orleans, LA 70112 (US).

(74) Agents: **BARZEE, Eric** et al.; Madson & Metcalf, 15 West South Temple, Suite 900, Salt Lake City, UT 84101 (US).

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(54) Title: NEUROPROTECTION BY INHIBITION OF DIACYGLYCEROL KINASE EPSILON ACTIVITY

(57) Abstract: The present invention includes the characterization of the DGKe gene and the generation of screening methods for compounds that inhibit the function of DGKe. The DGK family of enzymes occupies a signaling crossroads since they catalyze the phosphorylation of DAG to produce PA. Both the substrate (DAG) and the product (PA) of this reaction are key factors in intracellular signaling, making the regulation of DGKe activity important to understand and control. DGKe -/- mice were also generated and studied to assist in understanding the function of DGKs in regulating cellular signaling. DGKe displays selectively for 20:4-DAG and is highly expressed in different areas of the brain, including Purkinje cells in the cerebellum, hippocampal interneurons, and the Pyramidal neurons in the CA3 region of the hippocampus.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/08853

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A01K 67/00; C12Q 1/48; C12N 5/00

US CL : 435/15, 325; 800/13

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. : 435/15, 325; 800/13

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)

MEDLINE, AGRICOLA, CAPLUS, BIOSIS, EMBASE, WPIDS, EAST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	TANG, W. et al. Characterization of the human diacylglycerol kinase epsilon gene and its assesment as a candidate for inherited retinitis pigmentosa. Gene. October 1999, Vol. 239, No. 1, page 190.	23, 24 ---------- 25, 26
Y	TANG, W. et al. Molecular Cloning of a Novel Human Diacylglycerol Kinase Highly Selective from Arachidonate-containing Substrates. April 1996, Vol. 271, No. 17, pages 10238, 10240, and 10241.	1-5
Y	US 5,976,875 A (PRESCOTT et al.) 02 November 1999 (02.11.1999), column 10.	1-5
Y	OHANIAN et al. Lipid second messenger regulation: the role of diacylglyerol kinases and their relevance to hypertension. J Hum Hypertens. February 2001, Vol. 15, No. 2, see the whole document.	1-5, 12-22
X, P --- Y, P	RODRIGUEZ DE TURCO et al. Diacylglycerol kinase epsilon regulates seizure susceptibility and long-term potentiation through arachidonyl-inositol lipid signaling. Proc Natl Acad Sci USA. April 2001, Vol. 98, No. 8, see the whole document.	1-25 ---------- 26

☒ 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 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
19 August 2002 (19.08.2002)	19 SEP 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer David J. Steadman Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

BOX II. 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 I, claim(s) 1-5, drawn to a method of screening for agents that regulate DGK-epsilon activity by contacting a cell overexpressing DGK-epsilon with a test compound and measuring the level of DGK-epsilon activity in the cell in the presence of the test compound, wherein a compound that increases or decreases DGK-epsilon activity regulates DGK-epsilon activity.

Group II, claim(s) 6-11, drawn to a method of screening for agents that regulate DGK-epsilon activity by administering a test compound to an animal, administering a seizure stimulus to the animal, and measuring the level of DGK-epsilon activity in the animal, wherein a compound that increases or decreases DGK-epsilon activity in the animal regulates DGK-epsilon activity.

Group III, claim(s) 12-19, drawn to a method of screening for agents for treatment of a disorder by contacting a cell or an animal overexpressing a DGK-epsilon gene product with a test compound and measuring the inhibition of the DGK-epsilon gene product in the cell in the presence of the test compound.

Group IV, claim(s) 20-22, drawn to a method for inducing resistance to disorders by administering a compound that inhibits DGK-epsilon activity.

Group V, claim(s) 23-26, drawn to a transgenic nonhuman animal whose germ and somatic cells comprise at least one chromosome with a disruption to the endogenous DGK-epsilon gene and a cell derived from said transgenic nonhuman animal.

The inventions listed as Groups I-V 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: The methods of Groups I-V do not have unity of invention as the methods of Groups I-V comprise unrelated steps, utilize different products and/or yield different results.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/08853

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

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

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim 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. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 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.:

Remark on Protest

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

☐ No protest accompanied the payment of additional search fees.