available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Genetic interactions among cortical malformation genes that influence susceptibility to convulsions in *C. elegans*

Cody J. Locke[a,1], *Shelli N. Williams*[a,1], *Erich M. Schwarz*[b], *Guy A. Caldwell*[a], *Kim A. Caldwell*[a,*]

[a]*Department of Biological Sciences, The University of Alabama, Box 870344, Tuscaloosa, AL 35487-0344, USA*
[b]*Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA*

ARTICLE INFO

Article history:
Accepted 20 August 2006
Available online 22 September 2006

Keywords:
Epilepsy
Genetics
Lissencephaly
Caenorhabditis elegans
RNAi

Abbreviations:
RNAi, RNA interference
PTZ, pentylenetetrazole

ABSTRACT

Epilepsy is estimated to affect 1–2% of the world population, yet remains poorly understood at a molecular level. We have previously established the roundworm *Caenorhabditis elegans* as a model for investigating genetic susceptibilities to seizure-like convulsions in vivo. Here we investigate the behavioral consequences of decreasing the activity of nematode gene homologs within the LIS1 pathway that are associated with a human cortical malformation termed lissencephaly. Bioinformatic analysis revealed the *nud-2* gene, encoding the worm homolog of mammalian effectors of LIS1, termed NDE1 and NDEL1. Phenotypic analysis of animals targeted by RNA interference (RNAi) was performed using a pentylenetetrazole (PTZ) exposure paradigm to induce convulsions. Worms depleted for LIS1 pathway components (NUD-1, NUD-2, DHC-1, CDK-5, and CDKA-1) exhibited significant convulsions following PTZ and RNAi treatment. Strains harboring fluorescent markers for GABAergic neuronal architecture and synaptic vesicle trafficking were employed to discern putative mechanisms accounting for observed convulsion behaviors. We found that depletion of LIS1 pathway components resulted in defective GABA synaptic vesicle trafficking. We also utilized combinations of specific genetic backgrounds to create a sensitized state for convulsion susceptibility and discovered that convulsion effects were significantly enhanced when LIS-1 and other pathway components were compromised within the same animals. Thus, interactions among gene products with LIS-1 may mediate intrinsic thresholds of neuronal synchrony.

1. Introduction

Epilepsy affects over fifty million people worldwide and it is estimated that genetic factors account for the origins of ~40% of epilepsies (Noebels, 2003). Genetic epilepsies are often complex disorders in which only about 1% of patients show simple inheritance (Robinson and Gardiner, 2004). Lissencephaly is a developmental abnormality associated with a failure in the final migration event of neurons within the periventricular zone to the cortical plate; the majority of cases are caused by mutations or deletions of the *LIS1* gene. Patients often display intractable epilepsy that generally

* *Corresponding author*. Fax: +1 205 348 1786.
E-mail address: kcaldwel@bama.ua.edu (K.A. Caldwell).
URL: http://www.bama.ua.edu/~gcaldwel (K.A. Caldwell).
[1] These authors contributed equally to this work.

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.08.067

begins late in the first year of life (Dobyns, 1989; Miller, 1963).

Insights into the cellular functions of LIS1 have been elucidated through investigations of this protein within model organisms. Originally identified in *Aspergillus nidulans*, a LIS1 homolog termed NUDF was shown to have an essential role in nuclear distribution within hyphae of this filamentous fungus (Xiang et al., 1995). This mutation was termed *nud* for *nu*clear *d*istribution defective. There are also well-characterized *nud*/LIS1 pathway members in both higher and lower eukaryotic model organisms (Morris et al., 1998; Vallee et al., 2000). These gene products are highly conserved and function as a biochemical complex in multiple cell types, including migrating neurons and asymmetrically dividing cells.

The LIS1 protein is a member of the WD-40 repeat protein family (Neer et al., 1994), molecules characteristically involved in protein–protein interactions. Therefore, it is not surprising that many proteins have been identified as interacting partners of LIS1 (Emes and Ponting, 2001; Morris, 2000; Reiner, 2000). In multiple systems, LIS1 has been shown to interact with dynein, NUDC, and NUDE (Efimov and Morris, 2000; Faulkner et al., 2000; Gonczy et al., 1999a; Miyajima et al., 1995; Morris et al., 1998; Reiner, 2000; Smith et al., 2000; Vallee et al., 2000; Xiang et al., 1995). Dynein is a well-characterized motor protein while NUDC associates with the dynein motor complex and is a highly conserved protein of unknown function with roles in cell division and neuronal movement (Aumais et al., 2001, 2003). There are two mammalian homologs of NUDE, termed NDE1 and NDEL1. The NDEL1 protein associates with dynein heavy chain (Liang et al., 2004; Niethammer et al., 2000; Sasaki et al., 2000) and is also phosphorylated by Cdk5, a unique member of the cyclin dependent kinase family that is activated by p35 in neurons (Gilmore et al., 1998; Lew et al., 1994; Smith et al., 2000; Tsai et al., 1994). Loss of *Ndel1* gene activity in mice results in neuronal migration defects that are exacerbated by *Lis1* mutation (Sasaki et al., 2000). Mice lacking Cdk5 or p35 exhibit neuronal migration defects in which the normal inside-out neurogenic gradient of the cerebral cortex is reversed (Chae et al., 1997; Liu and Kipreos, 2000). Furthermore, p35 homozygous knockout mice display an increased number of lethal seizures compared to wild type siblings (Liu and Kipreos, 2000).

Despite evidence that the LIS1 pathway is involved in neuronal development, little is known about the relative importance of these cytoskeletal factors in the epileptic process. As the neuronal cytoskeleton interacts with synaptic trafficking components, neurotransmitter receptors and ion channels, disruption of these components can dramatically alter normal neuron function (Whatley and Harris, 1996). Given the complexity of the human brain and central nervous system, we contend that genetic analysis in a simple model organism may provide a more rapid means to discern the

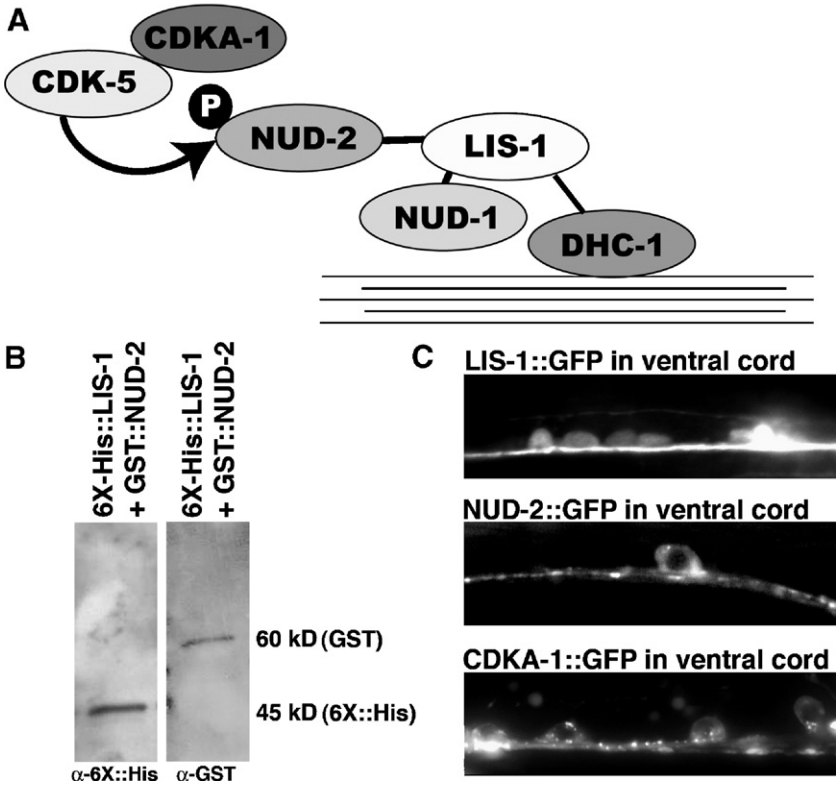

Fig. 1 – LIS-1 pathway components in *C. elegans*. (A) Simplified interaction pathway of LIS-1 and related proteins. Phosphorylation of NDEL1 (NUD-2 in *C. elegans*) by the p35/Cdk5 (*C. elegans* CDKA-1/CDK-5) complex facilitates its interaction with LIS-1, NudC (NUD-1) and subsequent regulation of dynein (DHC-1)-mediated microtubule processes. (B) Western blot showing in vitro interaction between 6X-HIS::LIS-1 and GST::NUD-2 following pulldown of these proteins using nickel sepharose. (C) In vivo expression of GFP translational fusions with LIS-1 (top), NUD-2 (middle) or CDKA-1 (bottom) in separate transgenic *C. elegans* lines. Expression of all three proteins is evident within the ventral nerve cord.