Doublecortin (DCX) is a microtubule-associated protein required for neuronal migration to the cerebral cortex. DCAMKL1 consists of an N terminus that is 65% similar to DCX throughout the entire length of DCX, but also contains an additional 360 amino acid C-terminal domain encoding a putative Ca\(^{2+}\)/calmodulin-dependent protein kinase. The homology to DCX suggested that DCAMKL1 may regulate microtubules, as well as mediate a phosphorylation-dependent signal transduction pathway. Here we show that DCAMKL1 is expressed throughout the CNS and PNS in migrating neuronal populations and overlaps in its expression with DCX and microtubules. Purified DCAMKL1 associates with microtubules and stimulates polymerization of purified tubulin and the formation of aster-like microtubule structures. Overexpressed DCAMKL1 leads to striking microtubule bundling in cell lines and cultured primary neural cells. Time-lapse imaging of cells transfected with a DCAMKL1-green fluorescent protein fusion protein shows that the microtubules associated with the protein remain dynamic. DCAMKL1 also encodes a functional kinase capable of phosphorylating myelin basic protein and itself. However, elimination of the kinase activity of DCAMKL1 has no detectable effect on its microtubule polymerization activity. Because DCAMKL1 is coexpressed with DCX, the two proteins form a potentially mutually regulatory network linking calcium signaling and microtubule dynamics.

Key words: neuronal migration; microtubule; kinase; microtubule-associated protein; doublecortin; lissencephaly
fectame (Qiagen, Chatsworth, CA) according to the manufacturer's recommendations. Cells were harvested 2 days later by adding boiling protein sample buffer (20 mM Tris, 5% glycerol, 0.625% SDS, and 5% β-mercaptoethanol) per plate (38 × 33 mm) in a boiling water bath and then homogenized using a Dounce homogenizer. The homogenate was centrifuged at 25,000 rpm at 35°C for 30 min. The supernatant and microtubule pellet were boiled in sample buffer and analyzed by Western blot to confirm the expression of PC2 in PC12. This method was run on an equilibrated Ni²⁺ column (Novagen, Madison, WI). The column was washed with 10 volumes of 1× binding buffer, six volumes of 1× wash buffer, then eluted with six volumes of 1× elution buffer, dialyzed, and concentrated to 1 mg/ml using a Centricon column (Amicon, Beverly, MA).

**Microtubule polymerization assays.** Assessment of aster formation was performed as previously described (Gleeson et al., 1999). Briefly, thymidine-labeled DCAMKL1 (Cytoskeleton, Denver, CO) was mixed with phosphocellulose-purified (PCP) tubulin (Cytoskeleton) to a final concentration of 0.5 mg/ml. DCAMKL1 (0.01–0.7 mg/ml) was added in BRBB80 buffer (in mM: 80 PIPES, pH 6.8, 1 M NaCl, and 1 M EGTA) in 1 mM GTP for 15 min at 37°C. Samples were fixed with 0.1% glutaraldehyde and 0.1% formalin in PBS. Aqueous solutions were then visualized with fluorescent microscopy.

For the quantitative analysis of microtubule polymerization, PCP tubulin (1 mg/ml) was added to specific concentrations of DCAMKL1, in 1 mM GTP, in PEM buffer. Each experimental sample was mixed briefly and assayed for microtubule polymerization using fluorescence microscopy (Gaskin et al., 1974). The kinetics of the diffraction for each sample was measured by right angle scattering in a quartz cuvette. The transmitted wavelength was 340 nm, and the detection wavelength was 345 nm, at a 90° angle to the transmitted beam, and the recording was performed for 16 min at 37°C in a Perkin-Elmer 202 (Ernest, CA). The initial diffraction was set to zero at time 0 for each sample.

**Construction of DCAMKL1-green fluorescent protein and kinase dead construct.** To create a green fluorescent protein (GFP)-labeled version of DCAMKL1, a CDNA fragment containing EGFP was cut out of the vector pEGFP-N1 (Clontech) using EcoR1 and PvuI digestion. The resulting 2.2-kb fragment was blunt-ended and then cloned into the pCMV-FLAG vector (Stratagene). Subsequently, the altered construct was checked for errors by sequencing through the open reading frame. Insertion of the EGFP-cDNA in the HindIII site was then performed as described above. Both NIH 3T3 and COS cells were transiently transfected with plasmids using Lipofectamine (Glycomatrix, San Diego, CA) and cultured for 2 d. COS cells were transfected with DCAMKL1-Myc for kinase assays and GFP analysis, whereas 3T3 cells were transfected for GFP analysis and time-lapse alone.

**In vitro kinase assays.** Imaging was performed essentially as described (M. Lu, L. Orecchio, and K. Kosik, unpublished observations). Cells transfected with DCAMKL1 constructs were examined using a Nikon Diaphot 300 microscope with 40× [1.0 numerical aperture (NA)] and 100× (1.4 NA) oil immersion lenses, with the GFP filter set (Chroma Tech). Fluorescence was quantitated using ImagePro Plus (Media Cybernetics, Silver Spring, MD), Adobe Photoshop, and Adobe Photoshop.

**Immunoprecipitation.** Control, DCAMKL1-Myc, and DCAMKL1 492-635-fused COS cells were prepared in 6-cm dishes as follows. Cells were washed once with ice-cold PBS and lysed (10 min on ice) in buffer H (in mM: 50 B-glycerophosphate, pH 7.3, 1.5 EGTA, 1 EDTA, 1 DTT, 0.1 sodium vanadate, 1 benzamidine, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 2 μg/ml pepstatin-A) (Seger et al., 1994). Cells were then scraped from the plate, pelleted, and homogenized using three strokes of a homogenizer. Cells were then centrifuged (14,000 × g; 20 min, 4°C). The supernatant was collected and incubated with 2 μg of anti-DCAMKL1 antibody (4°C) for 1 hr, then precipitated with protein A-agarose (Sigma) for 1 hr. Complexes were washed (4°C) three times with PBS + 0.1% NP-40, once with 0.1% LiCl in PBS (Pabico, Ames, IA), and once with ECL (ECL, Norcross, GA). A 50 μg B-glycerophosphate, pH 7.3, 1.5 EGTA, 1 EDTA, 1 DTT, and 0.1 NaVO₃) (Seger et al., 1994). Agarose beads were split into fractions and directly added to the kinase assay mixture or run on a Western blot.

Autophosphorylation and in vitro kinase assays. Kinase assays were performed as previously described (Lin et al., 2004). Briefly, 2 × 10⁶ NIH 3T3 cells were transfected with DCAMKL1-Myc (Promega, Madison, WI) plasmid DNA, and cell lysates were prepared as described above. The DCAMKL1-Myc phosphorylation was performed at 30°C in a buffer containing 50 mM HEPES, pH 8.5, 10 mM magnesium acetate, 10 μM leupeptin, 10 μM aprotinin, 50 mM B-glycerophosphate, 100 μM orthovanadate, 50 μM γ-[32P]ATP (20 cpm/μl) in a final volume of 30 μl. Reactions were initiated by adding 1 μg of the kinase substrate (20 μM adenosine 5′-triphosphate) with phosphocellulose-purified (PCP) tubulin (Cytoskeleton) and incubated with 0.5 μM of tubulin at 30°C. All reactions were run for 5 min. Reactions were terminated by adding 2× SDS-PAGE sample buffer and boiled for 5 min. All in vitro kinase assays were performed using the same mixture with different substrates. MBP was purchased from Sigma, and 0.06 μg was used per reaction.

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Lin et al. • DCAMKL1 Is an MAP and a Kinase

RESULTS

Characterization of polyclonal antisera specific to DCAMKL1

To determine the spatial and temporal expression of DCAMKL1, a polyclonal antibody was generated to a peptide immunogen corresponding to the N terminus of DCAMKL1. On a Western blot, the antibody recognized a major band at 80 kDa in whole-cell lysate from brain and a major band of slightly larger size in COS7 cells transfected with an epitope-tagged expression vector encoding full-length DCAMKL1 was probed with α-DCAMKL1. α-DCAMKL1 produces a specific band at 80 kDa both in cultured neurons and as a Myc-tagged fusion protein in overexpressing COS7 cells (90 kDa). The developmental regulation of DCAMKL1 in various aged human occipital cortex. Blots were probed with α-DCAMKL1 and subsequently probed with α-tubulin to control for protein loading. DCAMKL1 is expressed highly during embryonic life, then rapidly downregulated, with continued expression in the adult.

Spatial and temporal expression of DCAMKL1

To analyze the temporal and spatial pattern of DCAMKL1 expression further, a series of immunofluorescent stainings of mouse embryonic sections was performed (Fig. 2A–F). DCAMKL1 immunoreactivity at E14 was widespread throughout the nervous system. DCAMKL1 immunoreactivity was high in the developing retina (Fig. 2A), spinal cord and dorsal root ganglion (Fig. 2B), cerebellum (Fig. 2C), and cerebral cortex (Fig. 2D). Throughout all areas of the peripheral nervous system studied, such as the trigeminal ganglion, dorsal root ganglia (Fig. 2B), enteric plexus, and sympathetic ganglion, DCAMKL1 was expressed as well (data not shown). Furthermore, at P8, during migration of granule cells in the cerebellum (Fig. 2C), DCAMKL1 was expressed highly in areas that were rich in migrating granule cells, although the high levels of expression made it difficult to ascertain whether the staining was specific to migrating granule cells. There were lower levels of immunoreactivity in Purkinje cells and in the external granule layer. Comparison to the expression of DCX showed that DCAMKL1 and DCX are coexpressed in regions containing postmitotic, MAP2-positive migrating neurons (Fig. 2D–F), but low levels of DCAMKL1 immunoreactivity are also present in regions containing dividing cells such as the ventricular zone of the cortex (Fig. 3A–C). Thus, DCAMKL1 expression may not be completely neuron-specific, although studies of mRNA (Omori et al., 1998; Burgess et al., 1999) and protein localization using some other antisera and immunoperoxidase staining (Mizuguchi et al., 1999) showed lower levels of DCAMKL1 immunoreactivity in the cortical ventricular zone than observed with our antiserum. Within the developing cortex, DCAMKL1 expression was present at all ages examined. There was intense staining in the intermediate zone and cortical plate compared with the less intense ventricular zone staining at earlier stages (Fig. 3A,B) and more diffuse staining at later postnatal ages (Fig. 3C).

DCAMKL1 is present in radially migrating neurons in the cerebral cortex

Because DCAMKL1 is expressed highly by embryonic neurons that seem to be migrating radially in the intermediate zone and cortical plate, we performed confocal microscopy and stained individual radial migratory units to confirm that DCAMKL1 was indeed present in at least some radially migrating neurons (Fig. 3D–G). By using the cortical imprint method, individual radial migration units were isolated (Anton et al., 1999) and immunostained with a variety of cell type-specific antibodies. α-DCAMKL1 expression was present in the migrating neuron, whereas the glial fibers were immunoreactive for the monoclonal rat-401 antibody (Hockfield and McKay, 1985). Moreover, double-label confocal microscopy using the RC2 monoclonal antibody to stain radial glial fibers (Missen et al., 1988) confirmed that DCAMKL1 expression is present in bipolar profiles that strongly resemble migrating neurons (Fig. 3H–J). These data and previous data (Mizuguchi et al., 1999) suggest that DCAMKL1 is coexpressed in migrating neurons along with DCX (Gleseson et al., 1999), although DCAMKL1 may have some lower level of expression more broadly.

DCAMKL1 coassembles with microtubules from brain and leads to increased polymerization of microtubules

To define the subcellular localization of DCAMKL1, primary cortical neural cultures were immunostained with α-DCAMKL1. DCAMKL1 immunoreactivity was located predominantly along the length of cells with the morphological appearance of neurons (Fig. 4A). DCAMKL1 immunoreactivity was fibrillar under high power, suggesting an association with cytoskeletal elements (Fig. 4A; data not shown). Double-labeling of cells with antibodies directed against cytoskeletal proteins and α-DCAMKL1 revealed some overlapping localization with anti-α-tubulin antibodies (Fig. 4A–C). The colocalization was not absolute, but suggested the possibility that DCAMKL1, like DCX, may have areas of overlap with tubulin. In contrast, no consistent colocalization with actin or neurofilaments was observed (data not shown).

To examine whether the overlapping localization of DCAMKL1 with microtubules in cultured neurons reflected a functional interaction, microtubules from a cytosolic fraction of bovine brain were precipitated in the presence of taxol and analyzed for the presence of DCAMKL1. DCAMKL1 was substantially enriched in the taxol-stabilized pellet, whereas DCAMKL1 was not present in the pellet in the absence of taxol (Fig. 4D). A portion of DCAMKL1 (20%) remained in the supernatant in the presence of taxol as well, indicating that there may be distinct pools of DCAMKL1 not associated with microtubules. A MAP2C antibody was used as a
positive control for the procedure, showing that MAP2C was exclusively present in the Taxol-stabilized microtubule pellet fraction as expected.

Because of the homology of DCX with DCAMKL1, we next tested whether there are direct effects of DCAMKL1 on microtubule polymerization, similar to that observed for DCX (Gleeson et al., 1999), by incubating purified DCAMKL1 with rhodamine-labeled tubulin. Coincubation led to a dramatic stimulation of microtubule polymerization and to the formation of aster-like stars of polymerized rhodamine-labeled tubulin (Fig. 4E,F), suggesting that DCAMKL1, like DCX, can directly stimulate polymerization or stabilization of microtubules. To provide an estimate of the quantitative effects of DCAMKL1 in inducing microtubule polymerization, we used a turbidimetric assay (Gaskin et al., 1974) to compare the effects of DCAMKL1 and DCX. DCAMKL1 was HIS-tagged at both its N and C terminus (DCAMKL1 2HIS) or only at its C terminus end (DCAMKL1 1HIS). Equimolar quantities of either DCAMKL1 construct had very similar quantitative effects in this assay to DCX (Fig. 4G). We have previously shown (Gleeson et al., 1999) that the stimulation of microtubule polymerization by DCX occurs in the same order of magnitude of concentration as MAP2C, suggesting that DCAMKL1, like DCX, can stimulate microtubule polymerization at physiological concentrations.

Overexpression of DCAMKL1 leads to microtubule bundling and cold and drug stability

To determine whether DCAMKL1 can alter microtubule structure, Myc-tagged DCAMKL1 was over-expressed in primary cortical cultures and analyzed for effects on microtubules. Whereas cells overexpressing a negative control lacZ gene showed diffuse immunoreactivity and no effect on microtubules (Fig. 5A–C), overexpression of the epitope-tagged construct led to striking changes in microtubule morphology, with the development of rod-like bundles of microtubules throughout the transfected cells (Fig. 5D–F). Bundled microtubule patterns were quite common and observed in 70–90% of overexpressing cells; bundled patterns also tended to be more apparent and more complex in cells with very bright DCAMKL1 immunoreactivity as compared with untransfected neurons, although the elongated morphology of neurons may make microtubule bundling more difficult to detect.

Overexpression of other MAPs has been shown to lead to a relative stabilization of microtubules to drug and cold depolymerization (Takemura et al., 1992). We subjected DCAMKL1-transfected primary cortical cultures to cold treatment and colchicine treatment to examine whether DCAMKL1 overexpression also would stabilize microtubules to these treatments. After 2 hr of colchicine treatment or 30 min of cold treatment at 0°C, cells were then fixed with glutaraldehyde and processed to visualize DCAMKL1-Myc as well as microtubules. Treatment of transfected cells with colchicine led to a disruption of most microtubules and loss of some DCAMKL1-microtubule colocalization in cells expressing low levels of DCAMKL1 (Fig. 5G). However, transfected cells expressing higher levels of DCAMKL1 still maintained the
presence of some stabilized microtubules (Fig. 5G–I). A significant effect of DCAMKL1 on cold stability of microtubule bundles was also seen in transfected cells expressing high levels of DCAMKL1 (Fig. 5J–L) as compared with nontransfected cells in the same culture (data not shown). Cells transfected with DCAMKL1 maintained some bundles of microtubules after cold treatment when compared with neighboring nontransfected cells, which contained deteriorated microtubule structures.

**DCAMKL1 is a functional kinase**

Based on sequence homology, the primary structure of the C-terminus of DCAMKL1 encodes for a protein serine–threonine kinase similar to CaM kinase II. Previous reports have shown that CPG16, encoded by an alternative transcript that includes only the C-terminal domain of DCAMKL1, is a functional cAMP-dependent kinase that does not respond to calcium or calmodulin stimulation (Hervoni et al., 1998; Silverman et al., 1999). To determine whether full-length DCAMKL1 is a functional kinase, an in vitro kinase assay was performed. A Myc- or GFP-tagged construct containing the cDNA of DCAMKL1 was overexpressed in COS7 cells, and the DCAMKL1 fusion proteins were immunoprecipitated with α-DCAMKL1 antiserum. Precipitated DCAMKL1 was then used in an in vitro kinase assay with MBP protein. The results of the assay showed that both MBP and DCAMKL1 were phosphorylated by DCAMKL1 (Fig. 6, lane 2). Untransfected COS7 cell extracts subjected to immunoprecipitation showed some low background level of MBP phosphorylation (Fig. 6, lane 1).

An alternate trial involving the DCAMKL1-GFP construct showed a similar kinase activity to the Myc-tagged DCAMKL1 (Fig. 6, lane 3). A smaller band was present in some assays (Fig. 6, lanes 2, 3). This smaller product likely represents a phosphorylated cleavage product of DCAMKL1, because it was less apparent when the kinase assay was performed immediately after the immunoprecipitation and was more prominent when the kinase assay was performed several hours after the immunoprecipitation (data not shown). Contrary to previous results showing that CPG16 kinase activity was very low in the absence of 8-bromo-cAMP or forskolin (Silverman et al., 1999), full-length DCAMKL1 required no activation for functional kinase activity. Moreover, in our hands, full-length DCAMKL1 showed equivalent amounts of phosphorylation in the absence and presence of 8-bromo-cAMP (data not shown).

We created a point mutation in the DCAMKL1-GFP construct by site-directed mutagenesis of a previously identified site critical for kinase activity (Hanson and Schulman, 1992; Hanson et al., 1994). This mutated (K419R) DCAMKL1-GFP construct showed no ability to autophosphorylate or to phosphorylate MBP (Fig. 6, lane 4) above baseline.

**Kinase-independent effects of DCAMKL1-GFP on microtubule bundling**

Fusion of microtubule-associated proteins to the GFP allows for study of the real-time dynamics of cytoskeletal arrangements in cells in culture (Kaeche, et al., 1996). A DCAMKL1-GFP fusion construct was created to analyze the effect of DCAMKL1 on microtubule dynamics in real time. Expression of the GFP construct alone in transfected COS7 cells showed no effect on microtubule bundling (data not shown). Overexpression of the DCAMKL1-GFP construct showed strong colocalization and striking bundling of microtubules in a variety of patterns (Fig. 7A). In the majority of cells, microtubules appeared in bright bundles at the periphery of cells or in whorl-like spirals within the cell. All transfected cells showed microtubule bundling to some degree. Moreover, transfection of the DCAMKL1-GFP kinase-dead (K419R) construct showed no apparent difference versus the wild-type construct in the ability to bundle microtubules (Fig. 7B). Addition of latrunculin B, a potent actin depolymerization agent, led to the formation of processes containing bundled microtubules (Fig. 7C), confirming that the DCAMKL1 construct is localizing with microtubules (Edson et al., 1993; Matus, 1994; Kaeche et al., 1996).

Video time-lapse imaging of the DCAMKL1-transfected cells
DCAMKL1 is an MAP and a Kinase J. Neurosci., December 15, 2000, 20(24):9152–9161 9157

Figure 4. DCAMKL1 associates with microtubules and leads to microtubule polymerization. A–C, Subcellular localization of DCAMKL1 shows some localization that overlaps with microtubules. DCAMKL1 immunostaining of untreated E17 rat primary cortical neural cells (likely a neuron) demonstrates overlapping patterns of expression of DCAMKL1 with microtubules using α-tubulin antibody. DCAMKL1 and tubulin both were localized mainly to the processes of neurons. Scale bar, 10 μm. D, DCAMKL1 coprecipitates with taxol-stabilized microtubules from rat brain. Whole brain lysates from P5 newborn rat pups were cleared by centrifugation to isolate a tubulin-rich fraction and divided into two equal aliquots. To one aliquot, taxol and GTP were added and to the other aliquot, GTP alone was added, and microtubules were isolated by centrifugation. DCAMKL1 is enriched in the taxol-stabilized microtubule pellet (P, +Taxol), with smaller amounts remaining in the supernatant (S, –Taxol), whereas DCAMKL1 is not present in the pellet in the absence of taxol (P, –Taxol) and is retained in the supernatant (S, –Taxol). Reprobing a similar blot with α-pan-MAP2 indicates that DCAMKL1 distribution is similar to that of MAP2C–βC1. Whole-cell lysate; p, microtubule pellet. S, supernatant. E, F, Fluorescent images of rhodamine-tubulin plus DCAMKL1 shows expression predominantly of a truncated form that lacks the entire region of similarity to DCX and homology to human DCAMKL1, and may also be alternative splice forms of the same gene.

In their analysis of the kinase activity of CPG16, Silverman et al. (1999) found no stimulatory effect of calcium-calmodulin on kinase activity, and instead found stimulation of kinase activity by forskolin and 8-bromo-cAMP. They suggested that CPG16 may function as a β-AMP-dependent kinase and not as a CAM kinase, despite its lack of structural homology to βAMP-dependent kinases.

DISCUSSION

Here we show that full-length DCAMKL1 fulfills the criteria to be a member of a new family of microtubule-associated proteins, because it colocalizes with microtubules, coprecipitates with microtubules, and dramatically stimulates microtubule elongation when provided in purified form or transfected into cell lines or primary neural cells. The effects on microtubules do not require kinase activity, because they are retained in a kinase-dead protein. We also show that DCAMKL1 has kinase activity for itself, confirm that it has active kinase activity for MBP, and show that it can phosphorylate MBP in vitro without requiring stimulation. Finally, we show that DCAMKL1 is expressed in migrating neurons with DCX, suggesting that these two MAPs may function in concert to regulate microtubule dynamics in migrating neurons.

DCAMKL1 is expressed in the developing and adult brain as a number of alternative transcripts that include either its full-length, or else consist of only the amino terminal DCX-like region or the kinase-encoding C terminus alone (Omori et al., 1998; Burgess et al., 1999). The full-length transcript is most highly expressed during development (Omori et al., 1998), whereas the adult brain shows expression predominantly of a truncated form that lacks the entire region of similarity to DCX (Omori et al., 1998; Burgess et al., 1999). Intriguingly, the C-terminal splice form of DCAMKL1 had been previously cloned in rat as a candidate plasticity gene (CPG 16), defined as a transcript whose expression in the hippocampus is induced by the glutamate agonist kainic acid, suggesting a potential role in synaptic remodeling (Hevroni et al., 1998; Silverman et al., 1999). Two other recently cloned cDNAs induced by kainate (Vreugdenhil et al., 1999) or dopamine (Berke et al., 1998) stimulation also show regions of sequence identity to rat CPG16, and homology to human DCAMKL1, and may also be alternative splice forms of the same gene.

Microtubules are essential for the structural development of the nervous system, and are involved in the regulation of microtubule dynamics in migrating neurons. DCAMKL1 is expressed in migrating neurons with DCX, suggesting that these two MAPs may function in concert to regulate microtubule dynamics in migrating neurons.
and strong structural homology to CAM kinases. In contrast, we found readily evident levels of spontaneous kinase activity of DCAMKL1, and no stimulatory effect of 8-bromo-cAMP. Although we did not test effects of calcium-calmodulin on DCAMKL1, our results suggest that there are either technical differences in the assay, or that the full-length DCAMKL1 does not require 8-bromo-cAMP for its activity. Thus, lacking definitive evidence that full-length DCAMKL1 resembles a cAMP-dependent kinase, its structural homology to CAM kinases still warrants consideration that the full-length protein may show stimulation of its activity by Ca-calmodulin.

Our analysis is limited to full-length DCAMKL1. The full-length form is the predominant form expressed during the development of the cortex, whereas truncated isoforms such as CPG16 are persistently expressed in the adult (Omori et al., 1998). Our data are consistent with the DCX homology domain as being required for the MAP activity, and recent dissection of DCX using truncated constructs suggests that the microtubule binding domain is in the N-terminal 2/3 of DCX (Horesh et al., 1999). For example, missense mutations that disrupt neuronal migration (Gleeson et al., 1999), presumably because of loss of DCX function, all cluster in the N-terminal domain of DCX. These missense mutations occur in two evolutionarily conserved structural domains that have now been recognized in a number of molecules besides DCX and DCAMKL1, and these mutations disrupt the microtubule binding and polymerization activities of DCX (Sapir et al., 2000; Taylor et al., 2000). The effects of DCX missense mutations strongly suggest that interactions between DCX and tubulin are essential for normal neuronal migration. Therefore, the microtubule-binding domain of DCAMKL1 is likely to be encoded by the N terminus of DCAMKL1, which is highly homologous to the microtubule binding domain of DCX.

Microtubule function and neuronal migration

What is the role of microtubule reorganization in migrating neurons, and what role might DCAMKL1 play in this process? LIS1 shows a well conserved ortholog in Aspergillus nidulans called nudF (Xiang et al., 1995), whose role may represent a potential model for the role of LIS1, DCX, and potentially DCAMKL1 in neuronal migration. NudF is required for nuclear translocation along the fungal mycelium, and interacts genetically with genes that encode microtubule-related proteins such as α-tubulin and dynein (Willins et al., 1995, 1997; Morris et al., 1998). Nuclear translocation must also occur during the migration of neurons, and

Figure 5. Overexpression of DCAMKL1 in cultured neural cells leads to a microtubule bundling phenotype that is resistant to depolymerization with either cold or colchicine treatment. A–C, Transfection of neural cells (in this case a likely glial cell based on morphology) with lacZ-encoding vector leads to no change in microtubule bundling and a diffuse distribution of lacZ within the cytoplasm. D–F, Transfection of cells with DCAMKL1-Myc leads to microtubule bundling (F, arrows). Scale bar, 10 μm. G–I, Overexpression of DCAMKL1 stabilizes microtubules to colchicine treatment (H, I, arrows), whereas surrounding untransfected cells (data not shown) or cells expressing low levels of DCAMKL1 have destabilized microtubules. J–L, Microtubule bundles induced by overexpression of DCAMKL1 are partially resistant to cold (0°C) treatment (K, L, arrows), whereas microtubules in neighboring cells are disrupted (data not shown). Scale bars: A, D, G, J, 10 μm.
DCAMKL1-GFP transfected cells with MBP; MBP substrate; lane 2

the following assay mixtures were loaded: body was performed as described in Materials and Methods. Samples from

however, some lanes of the final gel are not illustrated for simplicity.

gel and developed on the same blot and same piece of film at the same time; were performed simultaneously and separated and transferred on the same

DCAMKL1. This degradation product was not seen in other experiments

with a shorter interval between reaction and loading and was more prom-

DCAMKL1 construct (4). Expression of DCAMKL1 as a Myc or GFP

fusional activity and strong kinase activity on MBP. Ten micrograms of MBP were used for each reaction. Some

phosphorylation of MBP is seen in untransfected COS cells (1). Kinase-dead

DCAMKL1 construct (4) construct. (4). Expression of DCAMKL1 as a Myc or GFP

fusion shows phosphorylation of DCAMKL1 and MBP, as well as an

identical ~40 kDa band that is probably a degradation product of

DCAMKL1. This degradation product was not seen in other experiments

with a shorter interval between reaction and loading and was more prom-

ter with a longer postreaction interval (data not shown). All reactions

were performed simultaneously and separated and transferred on the same
gel and developed on the same blot and same piece of film at the same time; however, some lanes of the final gel are not illustrated for simplicity.

the nucleus of migrating neurons shows a unique microtubule

structure surrounding it in the form of a “cage” (Rivas and Hatten,

1995). This perinuclear microtubule structure may be specialized

for translocating the nucleus into the leading process of the migrat-

ing neuron.

Because DCAMKL1 is a bifunctional molecule, with both MAP and kinase activities, its in vivo role may prominently reflect one of

the other of these functions, or may represent an interaction of the two. For example, DCAMKL1 (and DCX) may function primarily

as MAPs whose activity is regulated by phosphorylation. Phos-

phorylation is often a means for negatively regulating the interac-

tions of MAPs and microtubules (Raffaelli et al., 1992), raising the

possibility that phosphorylation of DCAMKL1 by itself or other

kinases may regulate its roles on microtubules. LIS1 preferentially

associates with microtubules in its dephosphorylated form (Sapir et

al., 1999), so that phosphorylation of DCAMKL1 may similarly

block its effects on microtubule stabilization. Neuronal migration to

the cortex is known to be saltatory, and is associated with calcium

transients, so that calcium transients could potentially give rise to

pulsatile cycles of microtubule stability and instability. The initial

phase of neuronal migration consists of neurite elongation, which is

associated with microtubule lengthening. The second phase of

migration involves neurite shortening. This process of neurite

shortening is complex and poorly understood, but presumably

requires rapid microtubule reorganization and perhaps shortening

or disassembly. This second phase is when nuclear translocation

takes place. Interestingly, the second phase of migration is associ-

ated with increases in intracellular calcium (Komuro and Rakic,

1992, 1996, 1998), suggesting that intracellular calcium may regu-

late specific stages of microtubule reorganization. Because CAM

kinases are activated downstream of intracellular calcium, phos-

phorylation of MAPs such as DCX and DCAMKL1 by

DCAMKL1 or other kinases could represent a rapid mechanism

for linking calcium transients to microtubule reorganization.

An alternative model suggests that DCAMKL1 plays a primary

signaling function through its kinase domain and that the kinase

activity of DCAMKL1 may be modulated by interactions with

microtubules, DCX, or both. Thus, DCAMKL1 shows baseline

autophosphorylation activity and strong kinase activity on MBP.

This kinase activity may be negatively regulated by truncation of

the N-terminal DCX domain of DCAMKL1, because CPG16, a

DCAMKL1 isoform that lacks the DCX domain, shows low levels

of unstimulated kinase activity (Silverman et al., 1999). Further

analysis of the primary amino acid structure of DCAMKL1 and

DCX revealed several highly conserved CAM kinase II consensus

phosphorylation sequences, when searched with the PhosphoBase

Figure 6. In vitro kinase activity of DCAMKL1 fusion proteins is blocked by a K419R point mutation. The various constructs of DCAMKL1 used in this study are labeled above the blot. COS7 cell extracts were transfected with either no construct (COS, 1), DCAMKL1-Myc (2), DCAMKL1-GFP (3), or the kinase-inactive K419R DCAMKL1-GFP (4) constructs. Transfection and immunoprecipitation with anti-Myc or anti-DCAMKL1 antibody was performed as described in Materials and Methods. Samples from the following assay mixtures were loaded: lane 1, nontransfected cells with MBP substrate; lane 2, DCAMKL1-Myc transfected cells with MBP; lane 3, DCAMKL1-GFP transfected cells with MBP; lane 4, K419R transfected cells with MBP. Ten micrograms of MBP were used for each reaction. Some phosphorylation of MBP is seen in untransfected COS cells (1) that is comparable with the level of phosphorylation seen in the kinase-dead DCAMKL1 construct (4). Expression of DCAMKL1 as a Myc or GFP fusion shows phosphorylation of DCAMKL1 and MBP, as well as an unidentified ~40 kDa band that is probably a degradation product of DCAMKL1. This degradation product was not seen in other experiments with a shorter interval between reaction and loading and was more prominent with a longer postreaction interval (data not shown). All reactions were performed simultaneously and separated and transferred on the same gel and developed on the same blot and same piece of film at the same time; however, some lanes of the final gel are not illustrated for simplicity.

Figure 7. Overexpression of GFP-tagged DCAMKL1 in COS7 cells and NIH 3T3 cells induces microtubule bundling with maintained microtubule dynamics (4). Transfection of COS7 cells with DCAMKL1-GFP leads to a striking amount of microtubule bundling (arrows). B, Transfection of COS7 cells with kinase-dead DCAMKL1-GFP K419R leads to no apparent difference in degree of microtubule bundling from that of wildtype construct. C, Addition of 0.2 μM Latrunculin B to COS7 cells transfected with DCAMKL1-GFP leads to the development of multiple processes containing bundled microtubules (arrows). D–H, Video frames taken from a time-lapse recording of a single NIH 3T3 cell (which show similar microtubule bundling effects to COS7 cells) showing DCAMKL1-GFP labeled microtubules and maintenance of microtubule dynamics. During this time, a single microtubule (arrow) is seen to collapse and reextend. Scale bars: A–C, 10 μm.
program (http://www.cbs.dtu.dk/hbts/pbase predict.pl). T-42 of


terated, microtubule-associated protein expressed in migrating


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