Doublecortin Kinase-2, a Novel Doublecortin-related Protein Kinase Associated with Terminal Segments of Axons and Dendrites*

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The microtubule (MT)-associated DCX protein plays an essential role in the development of the mammalian cerebral cortex. We report on the identification of a protein kinase, doublecortin kinase-2 (DCK2), with a domain (DC) highly homologous to DCX. DCK2 has MT binding activity associated with its DC domain and protein kinase activity mediated by a kinase domain, organized in a structure in which the two domains are functionally independent. Overexpression of DCK2 stabilizes the MT cytoskeleton against cold-induced depolymerization. Autophosphorylation of DCK2 strongly reduces its affinity for MTs. DCK2 and DCX mRNAs are nervous system-specific and are expressed during the period of cerebrocortical laminiation. DCX is down-regulated postnatally, whereas DCK2 persists in abundance into adulthood, suggesting that the DC sequence has previously unrecognized functions in the mature nervous system. In sympathetic neurons, DCK2 is localized to the cell body and to the terminal segments of axons and dendrites. DCK2 may represent a phosphorylation-dependent switch for the reversible control of MT dynamics in the vicinity of neuronal growth cones.

Mutations in the doublecortin gene DCX are responsible for cases of X-linked lissencephaly and doublecortex syndrome (for which the gene was named) (1–6). In these disorders the defective DCX protein fails to perform its normal embryonic function of mediating the migration of neuroblasts from the proliferative ventricular zone toward the pial surface. This results in disordered cortical laminiation, the absence (or paucity) of gyri and sulci, and a lissencephalic cerebral appearance. Recognition of DCX as a microtubule (MT)1-associated protein (MAP) therefore provided strong evidence for the critical role of MAP regulation of MT dynamics in neuronal migration (7–9). Patient mutations in X-lissencephaly/doublecortex syndrome and residues required for MT binding map to an N-terminal segment of DCX termed here, the DC domain (amino acids 43–259), and the homologous region in DCKs. In contrast, the C-terminal tail of DCX (268–C terminus), a region rich in Ser, Thr, and Pro residues (the SP domain), is without MT binding capability (10–12).

Independently, a protein kinase with an N-terminal segment homologous to DCX was described (13–19). Although its kinase domain is related to those of calmodulin (CaM)-dependent protein kinases, its catalytic activity is not regulated by CaM (19). Here, as in Shang et al. (19), it is referred to as doublecortin kinase-1 (DCK1),2 by virtue of its having a DC domain as a distinctive feature. Isoforms of DCK1 are generated by two mechanisms. Alternate promoter usage leads to protein products with identical kinase domains but either full-length (340 residues, α) or N-terminally truncated (31 residues, β) DC/SP domains. Consequently, DCK1-α binds MTs, whereas in DCK1-β, loss of the DC domain, leaving only a residual SP domain, results in an inability to bind MTs. Additional isoforms of DCK1 are produced by alternate splicing (13, 20, 21). DCK1 is encoded by a single gene mapped to human chromosome 13q13, and therefore is distinct from DCX on human chromosome Xq22.3–23. Although DCK1 is the only DCK gene described to date in vertebrates, predicted DC domain-kinase fusions are encoded by the genomes of Drosophila and Caenorhabditis elegans. The latter (ZYG-8) has been characterized and shown to be important for mitotic spindle positioning during C. elegans embryogenesis (22). We report here the identification and characterization of a novel mammalian DCK gene, DCK2, and its transcript and protein products. The patterns of expression, functional activities, regulation, and localization of DCK2 suggest that it functions in parallel to, or in concert with, other members of the DC gene family (DC domain-encoding genes) in events important for neural development and, potentially, in those characteristic of mature nervous systems.

**Experimental Procedures**

cDNA Cloning and Sequence Analysis of DCK2—cDNA was prepared from the brains of adult Sprague-Dawley rats by CsCl density gradient centrifugation (23). To obtain a probe for cDNA library screening, RT-PCR was performed using PCR primers based on the sequence of an EST (GenBankTM accession number W30246) encoding a putatively novel DCK (DCK is a protein kinase with a DC domain). Note that the term does not necessarily imply an nate; PIPES, 1,4-piperazinediethanesulfonic acid; nt, nucleotide; EGFP, enhanced green fluorescent protein.

2 DCK1 has also been referred to as CaMKL, cpg 16, DCaMKL1, DCLK, and KIAA0369.

* This work was supported by National Science Foundation Grants IBN0115449 (to A. M. E.) and IBN0121210 (to D. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡ The abbreviations used are: MT, microtubule; AP, alkaline phosphatase; DCK, doublecortin kinase; SDC, DCK2-(318–767); GAPDH, glyceraldehyde phosphate dehydrogenase; GST, glutathione S-transferase; HRP, horseradish peroxidase; KIN’, DCK2(α), HWM-MAP, high molecular weight (~250 kDa) MT-associated protein; PC, phosphocellulose; RT, reverse transcription; WT, wild type; CaM, calmodulin; CaMK, CaM kinase; MAP, microtubule-associated protein; DTT, dithiothreitol; PBS, phosphate-buffered saline; BSA, bovine serum albumin; RACE, rapid amplification of cDNA ends; FITC, fluorescein isothiocyanate; PIPES, 1,4-piperazinediethanesulfonic acid; nt, nucleotide; EGFP, enhanced green fluorescent protein.
ability to phosphorylate doublecortin). A 321-bp fragment was amplified, sequenced to confirm its identity, and biotinylated by random oligonucleotide-primer labeling using biotin-14-dATP as per the manufacturer’s instructions (PerkinElmer Life Sciences). A cDNA library was prepared from whole brain of Sprague-Dawley rats (10–12 weeks) in \( \lambda gt10 \) (Clontech) was screened using the biotinylated fragment described above as probe. Positive plaques were detected by streptavidin-HRP binding and ECL as per the manufacturer’s instructions (PerkinElmer Life Sciences), rescreened (plaque-purified) twice, and the inserts sequenced.

The library clones yielded 2.5 kb of overlapping sequence representing about two-thirds of the protein coding region, the stop codon, and all of the 3’-untranslated region including a polyadenylation signal (AATAAA) and poly(A) tail. An additional 5’ sequence was obtained as follows. 5’-RACE was performed by reverse transcription of rat brain RNA using a primer based on a 5’ sequence obtained from the library clones. First strand cDNA was DC-tailed and amplified with anchor and nested primers, re-amplified with an additional nested primer, and cloned in Escherichia coli. Sequencing of multiple independent clones extended the sequence another 168 bp toward the 5’-end. RT-PCR was then carried out using a reverse PCR primer based upon the 5’ sequence of RACE clones and a forward primer based on a rat EST sequence (GenBank accession number AA618566) overlapping with the RACE-generated sequence. The PCR products were purified with ammonia-gated anti-rat IgG (Molecular Probes); rat anti-pheromone binding protein (PBP)-conjugated anti-rabbit IgG (Roche Applied Science); and Texas Red-conjugated anti-mouse IgG and rhodamine-conjugated anti-mouse, -rabbit, or -goat IgG, Cy5-conjugated anti-mouse IgG, or anti-rat IgG (Jackson ImmunoResearch Laboratories). DCK2 was amplified from rat brain RNA and used to confirm the complete protein coding sequence.

Finally, RT-PCR of rat brain RNAs was performed to confirm the presence and translation of the start codon and 80 bp of 5’-untranslated region. Sequencing of the RACE clones and a forward primer based on a rat EST sequence (19) in 50 mM Tris-Cl, pH 8.0, 150 mM NaCl, 1% Triton X-100, 1% protease inhibitor mixture (Sigma), and protein phosphatase inhibitors (5 mM EDTA, 5 mM EGTA, 25 mM NaF, 1 mM orthovanadate, and 100 \( \mu \)M okadaic acid).

Primary cultures of sympathetic neurons were established as described previously (25). Briefly, superior cervical ganglia were isolated from E20–21 rats and treated with 2.5 mg/ml trypsin and 1 mg/ml collagenase for 40 min at 37 °C. The tissue was dissociated by trituration, and the neurons were plated onto coverslips coated with poly-lysine (100 \( \mu \)g/ml). Cells were maintained in serum-free medium containing \( \beta \)-nerve growth factor (100 ng/ml). Non-neuronal cells were removed by the addition of 1 \( \mu \)g/ml cytosine-\( \beta \)-D-arabinofuranoside for 48 h. Sympathetic neurons were co-transfected on the 5th or 6th day with pEGFP-N2 and pCDNA3.1-DCK2 using Lipofectamine 2000. After incubation for 6 h, cells were washed and allowed to recover for 24 h, following which they were grown in the absence or presence of 50 ng/ml bone morphogenetic protein (BMP)-7 (Cytoskeleton) as indicated.

**Microtubule Methods**—For co-assembly assays and for construction of MTs, the DCK2-K438A mutant (untagged or N-terminally FLAG-epitope tagged; where FLAG is an epitope tag with the sequence DYKDDDDK), respectively. DCK2K438A was cloned into pGEX4T-1 (Amersham Biosciences) for expression in E. coli (as a GST fusion protein) and into pcDNA3.1 (+) (Invitrogen) for expression in COS-7L cells or primary rat sympathetic neurons (untagged or N-terminally FLAG-epitope tagged; where FLAG is an epitope tag with the sequence DYKDDDDK), respectively. DCK2K438A (KIN1) was created by DpnI-based mutagenesis using Pfu Turbo DNA polymerase (Stratagene). DCK2-(318–767) (H9004) was covalently conjugated to activated CH-Sepharose 4B as per manufacturer’s instructions (Amersham Biosciences).

For assay of MT binding of COS cell-expressed DCK2, MTs were polymerized in vitro by incubation of 1 mg/ml tubulin (purified by phosphocellulose (PC) chromatography to remove endogenous MAPs) (Cytoskeleton) with either 30 \( \mu \)M taxol or vehicle (0.6% MeSO) in GPEM for 30 min at 30 °C. The MTs were mixed with lysates (3 \( \mu \)g protein) of COS-7L cells expressing the DCK2 constructs (WT or mutants) or a vector alone control in GPEM containing 150 mM NaCl and incubated for 15 min. The incubation mixtures were then centrifuged at 38,000 \( \times \) g for 30 min at 30 °C. The supernatant (S1) was retained, and the pellet was rinsed in GPEM/taxol, resuspended in this buffer, and centrifuged at 30,000 \( \times \) g for 30 min at 30 °C. The supernatant (S2) was retained, and the pellet (MT) was rinsed and then resuspended in GPEM. Purified, taxol-stabilized MTs were co-transfected into NIH 3T3 cells to generate plasmids containing full-length DCK2 cDNA, which were then further subjected to more complete MT polymerization by increasing the tubulin, taxol, pH values, and temperature to 2 mg/ml, 50 \( \mu \)M, 6.9 and 32 °C, respectively. The MTs were centrifuged as above at 32 °C, and in the binding reaction, the NaCl concentration was reduced to 100 mM.

**Phosphorylation Analysis and Peptide Kinase Assay—DCK2 auto-phosphorylation or high molecular weight microtubule-associated protein (HMW-MAP) phosphorylation reactions were at 30 °C for the periods of time and with ATP as indicated. Phosphorylation reaction buffer was 50 mM Tris, pH 7.6, 10 mM MgCl2, 0.5 mM DTT, and 1–2 mM EGTA. DCK2 dephosphorylation reactions were by incubation for 45 min at 30 °C with 50 units of calf intestinal alkaline phosphatase (AIP) (New England Biolabs) in a buffer consisting of 40 mM Tris, pH 7.9, 80 mM NaCl, 6 mM MgCl2, 0.7 mM DTT, and 8% glycerol. Dephosphorylation reactions were terminated with either SDS sample buffer for blotting applications or 10 mM NaPP, for MT-binding experiments. Peptide kinase activity of DCK2 (WT and mutants) was assayed after immunoprecipitation from lysates of transfected COS-7L cells by an immunocomplex assay as described previously, using the synthetic peptide LRRRLSASNP as substrate (19). Bacterial Expression and Purification of DCK2—GST-DCK2 was expressed in E. coli by induction of 0.2 mM isopropyl-\( \beta \)-D-thiogalactoside (IPTG) for 21 h at 15 °C. All subsequent procedures were performed at 4 °C. Cells were incubated with 0.5 mg/ml lysozyme in PBS containing protease inhibitors (1 \( \mu \)m phenylmethylsulfonyl fluoride, 20 mg/liter leupeptin, 20 mg/liter aprotinin, 2 mM EDTA), 0.5 mM DTT, and 1% Triton X-100 and lysed with a French pressure cell. The lysate was clarified by centrifugation, chromatographed batchwise on glutathione-Sepharose (Amersham Biosciences), and eluted with 10 mM reduced glutathione in 50 mM Tris, pH 8.0. The eluate was concentrated and dialyzed against 50 mM Tris, pH 7.5, 0.1 mM NaCl, 0.5 mM DTT, 1 mM EGTA, 1 mM EDTA, 0.05% Triton X-100, and 10% glycerol (dialysis buffer). It was then chromatographed on MT-Sepharose (prepared as described in Microtubule Methods) and dialyzed against GPEM/taxol and centrifuged at 30,000 \( \times \) g for 30 min at 30 °C. The supernatant (S1) was retained, and the pellet was rinsed in GPEM/taxol, resuspended in this buffer, and centrifuged at 30,000 \( \times \) g for 30 min at 30 °C. The supernatant (S2) was retained, and the pellet (MT) was rinsed and then resuspended in GPEM. Purified, taxol-stabilized MTs were co-transfected into NIH 3T3 cells to generate plasmids containing full-length DCK2 cDNA, which were then further subjected to more complete MT polymerization by increasing the tubulin, taxol, pH values, and temperature to 2 mg/ml, 50 \( \mu \)M, 6.9 and 32 °C, respectively. The MTs were centrifuged as above at 32 °C, and in the binding reaction, the NaCl concentration was reduced to 100 mM.

**Peptide Kinase Activity of FLAG-DCK2 (WT and mutants)**—Peptide kinase activity of FLAG-DCK2 (WT and mutants) was assayed as described (19) in 50 mM Tris-Cl, pH 8.0, 150 mM NaCl, 1% Triton X-100, 1% protease inhibitor mixture (Sigma), and protein phosphatase inhibitors (5 mM EDTA, 5 mM EGTA, 25 mM NaF, 1 mM orthovanadate, and 100 \( \mu \)M okadaic acid).
each time with 2.5 mg/ml sodium borohydride in 50% ethanol to reduce autofluorescence, and the borohydride was removed by washing three times for 5 min each time in PBS. Alternatively (as in Fig. 3B), the fixation and amplification procedure was modified as described by Rieder and Hard (27). Non-specific binding sites were blocked with 10% BSA in PBS for 3 h, followed by incubation with primary antibody in 3% BSA in PBS overnight. Cells were washed three times for 10 min each time with 0.1% Tween 20 in PBS and incubated in secondary antibody in 3% BSA in PBS for 30 min. Coverslips were rinsed successively with 0.1% Tween 20 in PBS and water and mounted using Slow Fade reagent (Molecular Probes).

For immunocytochemistry of primary sympathetic neurons, coverslip-attached cells were fixed in 4% paraformaldehyde in PBS for 15–20 min at room temperature, washed three times for 5 min each time with PBS, and permeabilized with 0.1% Triton X-100 in PBS for 3 min. After washing three times for 5 min each time with PBS, non-specific binding sites were blocked with 5% BSA in PBS for 2 h to overnight at 4 °C. Primary, and then secondary, antibody incubations were performed in this buffer overnight at 4 °C following which coverslips were rinsed in PBS and water and mounted in elvanol. In multiple labeling experiments, primary and secondary antibody incubations were performed sequentially.

Images were digitally acquired using a Bio-Rad MRC1024 laser scanning confocal head mounted on a Nikon Optiphot microscope equipped with a 100×/1.3NA (Fig. 8, B and C) and 100×/1.4NA (Figs. 3 and 4), 40×/H11003 (Fig. 3), and 60×/H9251 (Fig. 4) objectives equipped with objectives of 60×/H11003, 40×/H9251, and 20×/0.75NA (Fig. 8, B and C) and collected on separate fluorescence channels. Images were performed using Confocal Assistant (Bio-Rad) and Photoshop (Adobe). Immunoblotting using anti-DCK2 or anti-α-tubulin primary antibodies and HRP-linked secondary antibodies with ECL detection was performed as described previously (19).

Northern Hybridization Analysis—Probes were produced by PCR amplification of the following regions: DCX (nt 66–320; GenBank™ accession number BC056391) and DCK2 (kinase domain probe, nt 1968–2237; DC domain probe, nt 149–338; Fig. 1A). Northern blots with immobilized total RNA from staged mouse embryos and poly(A)+ RNA from selected mouse tissues were incubated and purified according to the manufacturer’s instructions (Stratagene). Northern blots with immobilized total RNA from staged mouse embryos and poly(A)+ RNA from selected mouse tissues were obtained from Seegene and Ambion, respectively. For the former, only lanes containing RNA from embryonic tissue without contribution from extra-embryonic RNA were analyzed. Blots were prehybridized in Ultrasolv (Ambion), to which 50 μg/ml denatured salmon sperm DNA was added, for 60 min at 42 °C. 32P-Labeled probe was briefly denatured, added to a concentration of 1.0 × 106 cpm/ml, and hybridized overnight at 42 °C. Washing to reduce non-specific background was optimized for each blot/probe combination. Typically, it was two times for 15 min each time in 2× SSC, 0.1% SDS at 42 °C, two times for 15 min each time in 0.1× SSC, 0.1% SDS at 42 °C, and one time for 15 min in the latter solution at 60 °C. Specifically bound probe was detected by autoradiography. Blots were stripped for reprobing by boiling in 0.1× SSC, 0.1% SDS.

RESULTS

Cloning and Sequence Analysis of DCK2—A BLAST search of GenBank™ identified an EST (accession number W30246) with 70% amino acid similarity to rat DCK1 suggesting that it might encode a novel DCK. The distribution of residues between this putative DCK family member and DCK1 was consistent with encoding of their respective cDNA's by distinct genes. The cloning strategy entailed cDNA library screening with a probe based on the W30246 EST sequence followed by 5'-RACE and RT-PCR. RT-PCR of adult rat brain RNA and the sequencing of independent clones was used to confirm the complete protein coding sequence and to obtain a cDNA clone for expression in functional studies.

The nucleotide and deduced amino acid sequence is given in Fig. 1A. The sequence is annotated with the positions of individual exons obtained by alignment of the cDNA sequence against rat genomic DNA using the BLAT (UCSC) and BLAST (NCBI) algorithms. The cDNA sequence is composed of 18 predicted exons spanning 129.8 kb of genomic DNA. As it is likely the sequence does not represent the entire transcription unit, the size of the gene and numbering of exons will presumably require subsequent readjustment. The rat gene maps to cytogenetic locus 2q34. Its human orthologue maps to 4q31.23 and therefore represents a novel gene, distinct from that of DCK1 at 13q13 (13, 15, 17). It is therefore designated DCK2.

Alignment of multiple, independent DCK2 cDNA clones with genomic DNA also revealed that two exons, E5a and E17a, are alternately spliced. Whereas the upstream alternate exon, 5a, is in-frame and when included yields a 16-amino acid insert (residues 320–335), the more 3' alternate exon, 17a, is out-of-frame, so that two possible C termini differing by 52-amino acid residues are predicted (Fig. 1A). By RT-PCR of adult rat brain RNA using flanking primer sets for the two exons, exon 5a-containing transcripts are more prevalent than those in which this exon is spliced out; and there are approximately equal levels of transcripts with, as without, exon 17a.3 Quantitative relationships between levels of these alternate transcripts and whether splicing occurs in a developmental and/or cell type-specific fashion remain to be determined. The isoform characterized here, and referred to as DCK2, is one in which exon 5a is included and 17a is excluded (transcript variant 2, GenBank™ accession number AY673998). (Fig. 1A).

From the amino acid sequence of DCK2, a number of functional domains may be predicted (Fig. 1B). At its N terminus, DCK2 demonstrates (over ~360 residues) a high degree of amino acid sequence identity to DCX (72%) and to the homologous region of DCK1 (78%), as shown by quantitative pairwise comparisons (Fig. 1B, inset) and multiple sequence alignment (Fig. 1C). DCK2 therefore is the third copy of the DC sequence to be recognized in vertebrates. The invertebrate C. elegans DCK, ZYG-8 (22) has equivalent amino acid sequence identity (45–47%) to DCX, and to the DCX-homologous regions of DCK1 and DCK2. ZYG-8 may therefore represent an ancestral gene from which the latter are derived by gene duplication event(s) and subsequent mutation.

Mutation of the DCX gene and consequent disruption of its ability to modulate MT dynamics is thought to underlie the neuronal migration deficits in X-lissencephaly/doublecortex syndrome. A number of missense and truncation mutations in DCX have been described in these patients (1–6). These mutations, as well as biochemical and structural studies, highlight the importance of these residues for control of MT dynamics and have defined the DC domain as bipartite, being composed of two tandem MT-binding subdomains (9–11). Of 26 patient missense mutations (spanning both domains), the homologous amino acid residues in DCK2 are identical to those in wild-type DCX in 22 missense mutations and conservatively substituted in the remaining 3 (Fig. 1C). This high degree of conservation suggested the potential MT binding functionality of the DCK2 DC domain.

The C-terminal tail of DCX is not involved in MT binding (10). This region, rich in Ser, Thr, and Pro residues, termed the SP domain, is also present in DCK1 and DCK2. ZYG-8 may therefore represent an ancestral gene from which the latter are derived by gene duplication event(s) and subsequent mutation.

3 A. M. Edelman, W.-Y. Kim, D. Higgins, E. G. Goldstein, M. Oberdoerster, and W. Sigurdson, unpublished data.
which is dependent upon alternate splicing of an exon in a developmental and brain region-specific fashion (21). An alternate exon of DCK2 (5a) is also located in its SP region, although, as noted above, whether splicing of this exon is subject to regulation has yet to be examined.

In addition to the homology of their DC domains, DCK2 and
DCK1 demonstrate significant sequence relatedness between the remaining segments of their primary structures. DCK2 has 65% amino acid sequence identity with DCK1-β2 (19), an isoform lacking a DC domain. Other than to DCK1, DCK2 is most closely related to members of the CaMK subfamily, such as CaMKI (52% amino acid similarity). Within the predicted kinase domain of DCK2, a catalytic core, defined by sequence comparison to members of the main protein kinase superfamily, can be recognized (amino acid residues 409–666). Based on the conservation within this region of all of the residues thought to be required for protein Ser/Thr kinase activity (31), DCK2 may be predicted to be a catalytically active protein kinase. Its C-terminal tail of 49 or 101 residues (depending on variable inclusion of exon 17a) does not contain predicted functional domains. However, in an analogous fashion to CaMKI (32), the C-terminal tail of DCK1-β2 contains an autoinhibitory domain, the truncation of which markedly activates the enzyme (19). This observation was confirmed by Engels et al. (33) who also reported that C-terminal splicing of DCK1-β was capable of modulating its kinase activity. Thus, it is possible that for DCK2 variable inclusion of alternate exon 5a in the SP region, 17a in the C-terminal tail, or transient signals targeting either of these two regions could serve to regulate its kinase activity.

DCK2 Binds and Stabilizes MTs—We assessed the predicted MT binding activity of DCK2. First, a co-assembly assay was used to test whether endogenous brain DCK2 is capable of binding MTs polymerized from endogenous brain tubulin. An antipeptide antibody was prepared to its unique C terminus and recognized DCK2 at its predicted native Mr (84 kDa) by assuming inclusion of exon 5a and exclusion of exon 17a (transcript variant 2). Exon 17a is included because it would produce a distinct C terminus, not recognizable by the antibody which is C-terminally directed.
protein being enriched in the MT fraction by 32-, 60-, and 160-fold relative to the S1, S2, and S3 fractions, respectively. As a control, we determined the distribution of a member of the CaM kinase subfamily. In the co-assembly procedure, CaM kinase α (34, 35) showed no preferential association with MTs.3

We then assayed the ability of DCK2 to bind to MTs assembled from PC-purified tubulin in vitro. COS cell-expressed wild-type (WT) FLAG-DCK2 strongly bound to newly polymerized MTs (Fig. 2B). To investigate the role of the DC domain in MT binding, we truncated the N-terminal 317 amino acid residues of DCK2. FLAG-DCK2-(318–767) (ΔDC) demonstrated no ability to bind to MTs, thereby establishing the site of interaction as the DC sequence. We also asked whether a functional kinase domain is required for the MT binding ability of DCK2. In this assay, a KIN2Δ DC mutant (described below) was competent to bind to MTs. Moreover, the relatively low concentration of MTs that polymerize at 30 °C in the absence of taxol tends to bind a higher fraction of KIN2Δ DC compared with WT (Fig. 2B), suggesting that the former has a greater MT binding affinity than the latter. This difference is potentially explainable by WT DCK2 autophosphorylation during expression reducing its MT binding affinity. In sum, the results of Fig. 2 demonstrate that DCK2, either endogenous or recombinant, binds MTs and that the DC domain, but not the catalytic activity of DCK2, is required for MT binding.

We proceeded to investigate the localization of DCK2 and its capacity to interact with MTs in intact cells. COS-7L cells were transfected with nonepitope-tagged DCK2 (to avoid any possible redistribution because of the heterologous sequence), and its distribution was assessed by confocal microscopy using DCK2 immunofluorescence. Although endogenous DCK2 was not detected in the COS-7L kidney cell line, transfected DCK2 demonstrated a prominently fibrillar appearance (Fig. 3A). The appearance of both fine and thicker filaments resembling MTs, including some parallel and perpendicular to a ruffled lamellipodium (36), is consistent with DCK2 binding to MTs and their possible stabilization through the formation of bundles. By double labeling, MT immunostaining in DCK2-transfected cells demonstrated a very similar fibrillar appearance (Fig. 3B, middle versus left panel). Overlay of the images revealed a strong co-localization between DCK2 and MTs (Fig. 3B, right panel).

To ascertain whether DCK2 interaction with MTs in intact cells is mediated by its DC domain, we expressed and localized the ΔDC mutant. Absence of a DC domain completely abolished the fibrillar appearance of DCK2 immunofluorescence leading instead to diffuse cytosolic staining (Fig. 3C, upper panel). Moreover, the MT cytoskeleton in the ΔDC-transfected cells demonstrated a more typical appearance with little evidence of MT bundling, other than the intercellular bridges of cells completing cytokinesis (Fig. 3C, lower panel). Finally, we asked whether DCK2 expression could stabilize intracellular MTs. In cells transfected with full-length DCK2 and fixed at 4 °C to induce MT depolymerization, prominent MT bundles were seen by both DCK2 and α-tubulin immunostaining (Fig. 4, A and B). In contrast, transfection with ΔDC did not protect MTs against cold-induced depolymerization (Fig. 4, C and D). It may be concluded from the results of Figs. 2–4 that DCK2 associates with and stabilizes MTs and that these activities are mediated through its DC domain.

**Protein Kinase Activity of DCK2 and Regulation of MT Binding by Autophosphorylation**—As the DC domain of DCK2 is functional when fused to its kinase domain, we addressed the converse question of whether the kinase domain is catalytically competent when fused to the DC domain. DCK2 autophosphorylates in a time-dependent fashion in vitro. Autophosphorylation was observed both after bacterial expression as a GST fusion and purification by sequential glutathione- and MT-Sepharose chromatographies (Fig. 5A) and after expression as a hexahistidine fusion and affinity purification by Ni2+-agarose chromatography.3 Whether the mode of this autophosphorylation is intra- or intermolecular is unknown at present but may be addressed through future kinetic experiments. DCK2 is also capable of phosphorylating exogenous substrates, for example a HMV-MAP, in addition to autophosphorylating (Fig. 5B).

We also evaluated the potential kinase functionality of DCK2 expressed in mammalian cells. COS cell-expressed FLAG-DCK2 was immunoprecipitated using M2-anti-FLAG-Sepharose, and its ability to both autophosphorylate and phosphorylate an exogenous substrate was evaluated in immune complex assays (Fig. 5, C and D). Additionally, we mutated the “catalytic” Lys residue (Lys-438) (31) to an Ala and expressed protein being enriched in the MT fraction by 32-, 60-, and 160-fold relative to the S1, S2, and S3 fractions, respectively. As a control, we determined the distribution of a member of the CaM kinase subfamily. In the co-assembly procedure, CaM kinase α (34, 35) showed no preferential association with MTs.3

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We proceeded to investigate the localization of DCK2 and its capacity to interact with MTs in intact cells. COS-7L cells were transfected with nonepitope-tagged DCK2 (to avoid any possible redistribution because of the heterologous sequence), and its distribution was assessed by confocal microscopy using DCK2 immunofluorescence. Although endogenous DCK2 was not detected in the COS-7L kidney cell line, transfected DCK2 demonstrated a prominently fibrillar appearance (Fig. 3A). The appearance of both fine and thicker filaments resembling MTs, including some parallel and perpendicular to a ruffled lamellipodium (36), is consistent with DCK2 binding to MTs and their possible stabilization through the formation of bundles. By double labeling, MT immunostaining in DCK2-transfected cells demonstrated a very similar fibrillar appearance (Fig. 3B, middle versus left panel). Overlay of the images revealed a strong co-localization between DCK2 and MTs (Fig. 3B, right panel).

To ascertain whether DCK2 interaction with MTs in intact cells is mediated by its DC domain, we expressed and localized the ΔDC mutant. Absence of a DC domain completely abolished the fibrillar appearance of DCK2 immunofluorescence leading instead to diffuse cytosolic staining (Fig. 3C, upper panel). Moreover, the MT cytoskeleton in the ΔDC-transfected cells demonstrated a more typical appearance with little evidence of MT bundling, other than the intercellular bridges of cells completing cytokinesis (Fig. 3C, lower panel). Finally, we asked whether DCK2 expression could stabilize intracellular MTs. In cells transfected with full-length DCK2 and fixed at 4 °C to induce MT depolymerization, prominent MT bundles were seen by both DCK2 and α-tubulin immunostaining (Fig. 4, A and B). In contrast, transfection with ΔDC did not protect MTs against cold-induced depolymerization (Fig. 4, C and D). It may be concluded from the results of Figs. 2–4 that DCK2 associates with and stabilizes MTs and that these activities are mediated through its DC domain.

**Protein Kinase Activity of DCK2 and Regulation of MT Binding by Autophosphorylation**—As the DC domain of DCK2 is functional when fused to its kinase domain, we addressed the converse question of whether the kinase domain is catalytically competent when fused to the DC domain. DCK2 autophosphorylates in a time-dependent fashion in vitro. Autophosphorylation was observed both after bacterial expression as a GST fusion and purification by sequential glutathione- and MT-Sepharose chromatographies (Fig. 5A) and after expression as a hexahistidine fusion and affinity purification by Ni2+-agarose chromatography.3 Whether the mode of this autophosphorylation is intra- or intermolecular is unknown at present but may be addressed through future kinetic experiments. DCK2 is also capable of phosphorylating exogenous substrates, for example a HMV-MAP, in addition to autophosphorylating (Fig. 5B).

We also evaluated the potential kinase functionality of DCK2 expressed in mammalian cells. COS cell-expressed FLAG-DCK2 was immunoprecipitated using M2-anti-FLAG-Sepharose, and its ability to both autophosphorylate and phosphorylate an exogenous substrate was evaluated in immune complex assays (Fig. 5, C and D). Additionally, we mutated the “catalytic” Lys residue (Lys-438) (31) to an Ala and expressed...
and immunoprecipitated the mutant in parallel to WT. FLAG-DCK2 WT but not FLAG-DCK2K438A was autophosphorylated, consistent with the latter being KIN−/H11002 and with DCK2 having intrinsic protein kinase activity (Fig. 5C). Finally, kinase activity of FLAG-DCK2 was assayed using a synthetic peptide LRRRLSLANF as substrate. This peptide was used because it is kinetically favored by the related kinase DCK1-2/H9252 (19). WT FLAG-DCK2 phosphorylated this peptide, whereas the DCK2K438A mutant was inactive (KIN−), again consistent with DCK2 having intrinsic kinase activity (Fig. 5D). Furthermore, the ΔDC mutant had an equivalent level of peptide kinase activity as WT, indicating that the kinase domain is not dependent upon the DC sequence for expression of its catalytic activity. From the lack of activation of ΔDC relative to WT, it may be further inferred that residues within the DC domain do not repress the kinase activity of the catalytic domain, i.e. the DC domain does not harbor an autoinhibitory subdomain. Together, these results indicate that the protein kinase domain of DCK2 is functional and independent of the DC domain.

Although the results of Figs. 2 and 5 are consistent with the functional independence of the DC and protein kinase domains, we hypothesized that the two domains might nonetheless be able to cross-talk. This hypothesis was based on two observations. First, as noted above, COS cell-expressed KIN−/H11002 DCK2 had a somewhat greater tendency to bind MTs than did WT (Fig. 2B). Second, when GST-DCK2 expression was induced in E. coli over a prolonged period (at a low temperature to minimize proteolysis), the glutathione-Sepharose-purified enzyme appeared to be a poorly resolved doublet, both bands of which were immunoreactive with the C-terminally directed DCK2 antibody (Fig. 6, A and C). Given its capacity to autophosphorylate, we reasoned that DCK2 may have partially autophosphorylated during induction creating phospho and dephospho forms of differing electrophoretic mobilities. We attempted to separate these hypothetically phospho and dephospho isoforms by chromatographing the glutathione-Sepharose-purified enzyme on a column of taxol-stabilized MTs conjugated to Sepharose. This step effectively resolved the two bands of the doublet (Fig. 6A). The slow migrating band eluted from MT-Sepharose at a low (50 mM) NaCl concentration, whereas the rapidly migrating species was more tightly bound, eluting from the column with 600 mM NaCl. Electrophoresis of the pooled fractions and immunoblotting with the N-terminally directed GST antibody also demonstrated the ability of MT-Sepharose to...
effectively separate forms of DCK2 with differing electrophoretic mobilities (Fig. 6B).

At this point we asked whether, as hypothesized, differential phosphorylation was the cause of the electrophoretic mobility changes correlating with differing binding affinities to MT-Sepharose. Upon dephosphorylation by treatment with AP, the mobility of the slow migrating form of DCK2 was shifted to that of the rapidly migrating form, whereas the mobility of the latter was unaffected by phosphatase treatment (Fig. 6C). Conversely, incubation of the rapidly migrating form with ATP led to regeneration of the doublet consistent with partial autophosphorylation, whereas incubation of the slowly migrating form with ATP did not create a doublet (Fig. 6D). In the latter case, a slight apparent change in mobility was evident (Fig. 6D, compare lanes 3 and 4), possibly reflecting the potential for the in vivo phosphorylated form to autophosphorylate at additional sites in vitro. Together these results demonstrate a correlation between the phosphorylation state of DCK2 and its ability to bind to MT-Sepharose; the slowly migrating electrophoretic form of DCK2 was phosphorylated and this phosphorylation had the effect of reducing its affinity for MTs.

To evaluate this hypothesis further, we tested whether manipulation of the phosphorylation state of DCK2 in vitro could reversibly modulate its affinity for MTs. Initially, we observed that incubation with AP reversed not only the electrophoretic mobility change but also to a significant extent the weak binding of phospho-DCK2 to MT-Sepharose. By contrast, MT-Sepharose binding of dephospho-DCK2 was unaffected by phosphatase treatment. 3 To test the hypothesis by an independent method, we next conducted experiments using MTs polymerized from PC-purified tubulin subunits in vitro. For this series of experiments, the MT binding assay was modified (see under “Experimental Procedures”) to more consistently drive all free tubulin subunits to polymerize and thereby provide a stringent test of the ability of phosphorylation to release DCK2 from MTs. First, phospho and dephospho forms of DCK2 were analyzed for MT binding after incubation in the presence and absence of AP. Phospho-DCK2 was virtually completely unbound even at a high concentration of MTs (Fig. 6E, left panel). Phosphatase incubation produced a significant shift toward MT binding, with the proportion-bound roughly proportional to the MT concentration (see ± taxol conditions). By contrast, dephospho-DCK2 was overwhelmingly bound to MTs even at low MT concentrations, and the binding was unaffected by phosphatase treatment (Fig. 6E, right panel).

Having found an increase in MT binding upon dephosphorylation of DCK2, we asked the converse question of whether phosphorylation of dephospho-DCK2 could decrease MT binding. Dephospho-DCK2 was incubated under phosphorylation reaction conditions in the presence of a low concentration (20 μM) of either nonradioactive ATP or [γ-32P]ATP, and its binding to MTs was determined (Fig. 6F). At this low ATP concentration, the overall distribution as assessed by immunoblotting showed DCK2 still largely bound to MTs, although the relatively small percentage that underwent a mobility shift, and was therefore phosphorylated, was unbound (top panel). Significantly, when phosphorylated DCK2 was selectively monitored by 32P labeling and autoradiography, the phospho-DCK2...
showed a pronounced reduction in its ability to bind to MTs (Fig. 6F, middle panel). Overall, the data of Fig. 6 demonstrate reversible regulation by phosphorylation/dephosphorylation of DCK2 binding to MTs. These results suggest that autophosphorylation could represent a mechanism by which DCK2-MT interaction is modulated in vivo in response to intracellular signaling events that result in DCK2 activation.

It is also noteworthy that incubation of tubulin in the presence of 1 mM GTP at 30–32 °C leads to some MT polymerization even in the absence of taxol. The avid binding of KIN/H11002 and dephospho-DCK2 to MTs formed in the absence of taxol (Figs. 2B and 6E-right panel) has two implications in addition to supporting the hypothesis that DCK2 autophosphorylation regulates MT binding affinity. First, it implies that the ability of DCK2 to bind to MTs is not dependent upon taxol as a means of inducing polymerization. Second, DCX is known to bind unpolymerized tubulin in addition to MTs (11). Whether this is true for DCK2 as well will require further experimen-
Doublecortin Kinase-2

Developmental and tissue-specific expression of DCX and DCK2 mRNAs. A, Northern blot with immobilized total RNA from mouse embryo harvested at the indicated days post-coitum (pc) (E10.5–18.5) was hybridized, developed, stripped, and reprobed with probes specific for DCX (top panel), DCK2 (middle panel), and GAPDH (bottom panel). The DCK2 probe was directed toward the kinase domain. The positions of the 9.5-kb DCX and 4.5-kb DCK2 transcripts are indicated by arrows. B, Northern blot with immobilized poly(A)⁺ RNA from individual mouse tissues (8–10 weeks) or whole embryo was hybridized, developed, stripped, and reprobed with probes specific for DCX (top panel), DCK2 (middle panel), and GAPDH (bottom panel). The DCK2 probes were directed toward the kinase domain (KD) or DC domain as indicated. Tissues as indicated are: He, heart; Br, brain; Li, liver; Sp, spleen; Ki, kidney; Em, 14-day embryo; Lu, lung; Th, thymus; Te, testes; Ov, ovary. Note that the ~1.5-kb spot in the thymus lane of the top panel is artifactual.

As befits its ability to mediate embryonic neuronal migration, DCX is expressed in a nervous system-specific pattern and, although strongly expressed at E18.5, is subsequently repressed in the postnatal period (2, 7, 8, 39). This pattern is illustrated in Fig. 7B. DCX mRNA is expressed at detectable levels only in adult mouse brain and whole embryo (E14), with the embryo having much higher expression. In similar fashion to DCX, hybridization with DCK2-specific probes (Fig. 7B, middle panels) revealed detectable transcript levels only in brain or whole embryo. In sharp contrast to DCX, however, DCK2 mRNA is strongly expressed in adult brain relative to 14-day embryos, suggesting that DCK2 (at least in the mature mammalian nervous system) is not functionally redundant with DCX.

For DCK1, utilization of the promoter generating the DC domain-containing transcript (α) decreases postnataally in a manner similar to that of DCX (13, 18, 39). By contrast, the DCK1-β transcript that does not encode a DC domain is expressed at high levels in the adult. These expression patterns raised the possibility that the role of the DC sequence in any of its forms is, for the most part, temporally restricted to the period of embryonic development. We therefore tested whether the DC domain of DCK2 continues to be incorporated into mature transcripts after embryogenesis is complete. Northern hybridizations with two DCK2-specific probes, one directed to the kinase domain (KD probe) and the other to the DC domain (DC probe), yielded essentially identical expression patterns, both with respect to transcript size and tissue distribution (Fig. 7B, middle panels). These data as well as the cloning of DC domain-containing DCK2 cDNAs from adult brain indicate that there is at least one copy of the DC sequence (DCK2) persisting in abundance into adulthood and that the DC sequence therefore has role(s) unrelated to embryonic development.

DCK2 Localization in Sympathetic Neurons—MAPs such as MAP2, tau, and DCX itself are distributed to specific subcellular compartments within neurons (40–41). We therefore sought to probe the subcellular distribution of DCK2. Sympathetic neurons isolated from neonatal rat superior cervical ganglia offer an attractive system for this purpose because they can be maintained in culture at a high state of purity and at relatively low density, such that sorting of protein components to axons and dendrites can be clearly observed (25). Unlike COS cells that are non-neural in origin and do not express endogenous DCK2, sympathetic neurons might be expected to endogenously express DCK2. However, we have been unable to observe endogenous DCK2 in these neurons, suggesting that the antibody used here, although capable of detecting endogenous DCK2 in whole brain lysates (Fig. 2A), is of insufficient affinity to detect what might be a lower concentration of DCK2 in this specific neuronal population. Accordingly, we transfected sympathetic neurons with DCK2 and co-transfected EGFP to visualize processes. The distributions of DCK2, EGFP, and markers of the MT cytoskeleton, either tubulin or MAP2, were then studied by multilabel immunofluorescence confocal microscopy.

In contrast to the uniform distribution in both cytoplasm and nucleus of EGFP, DCK2 localized to a subplasmalemmal ring of MTs in the somal cortex (Fig. 8A), a pattern suggestive of MT...
 bundling, consistent with its distribution in COS cells (Figs. 3 and 4). In addition to its co-localization with MTs in the cell body, DCK2 was observed in the distal-most portions of axons in the vicinity of their growth cones (Fig. 8B). In dendrites, DCK2 was also found distal to the cell body (Fig. 8C) and again with staining in the subplasmalemmal cortex. This localization was clearly distinct from that of EGFP that was evenly distributed throughout the cell and was difficult to discern in relatively fine distal dendritic processes. Most interestingly, the distribution of DCK2 was also distinguished from that of endogenous MAP2 that was more uniformly distributed in the cell body and found in somewhat more proximal dendritic segments. Moreover, the targeting of DCK2 to both axons and dendrites is also distinct from that of MAP2, which is somatodendritic (41).

**DISCUSSION**

In this report we identify and characterize a novel DC and protein kinase gene and its protein product, DCK2 (Fig. 1A). DCK2 is composed of two functional and independent domains, an MT-binding and -stabilizing domain (the DC sequence) and a kinase domain with protein phosphotransferase activity. This domain organization is supported by the following evidence.

The DC domain of DCK2 is evolutionarily related, although nonidentical, with other DC-containing proteins such as DCX, the protein product of the gene mutated in X-lissencephaly/doublecortex syndrome (Fig. 1, B and C). In these disorders, patient mutations have been identified in DCX amino acids that are highly conserved with the homologous residues within the DC domain of DCK2 (Fig. 1C). This observation suggested the likely functionality of the DC domain of DCK2, and it is shown here through the following experimental observations. WT DCK2 associates with MTs in co-assembly and in vitro MT binding assays and also binds MT-Sepharose in an ionic strength-dependent fashion (Figs. 2 and 6). When expressed in COS cells and sympathetic neurons, it co-localizes with MTs, leads to a phenotype consistent with MT bundling, and stabilizes MTs against cold-induced depolymerization (Figs. 3, 4, and 8A). The ability of DCK2 to associate with MTs in vitro or associate with and stabilize MTs in cells is abolished by truncation of the N-terminal 317 amino acids, thereby assigning these functions to the DC domain (Figs. 2–4). Moreover, these MAP-like functions of the DC domain do not require the catalytic activity of the kinase domain because DCK2 made KINH/H11002 by mutation of a single residue (K438A) in its putative ATP-binding subdomain (Fig. 5, C and D) binds tightly to MTs (Fig. 2B). Similarly, a kinase-negative DCK1 has also been shown to be capable of binding MTs in vitro (43), although it should be noted that whether DCKs are capable of regulating MT dynamic instabil-
ity in vivo in the absence of kinase activity has yet to be rigorously tested.

One implication of the ability of the DC domain of DCKs to function independently of the kinase domain is that it would allow DCX itself to evolve through duplication of an ancestral DCK, followed by mutational loss of its kinase domain, an hypothesis consistent with the existence in the nematode C. elegans of a DCK (ZYG-8), but apparently no DCX, orthologue. From gene knock-out and RNA interference studies in rodents and the genetics of lissencephaly in humans, it is evident that DCX has acquired the specialized role of a mediator of neuron migration (2, 3, 37, 44). The ability of DCX to fulfill this function without a fused kinase domain raises the question of why the kinase domain has been retained in DCK2 and DCK1-α in the course of vertebrate evolution. One possibility is that the kinase activity of DCK2 had been disabled by mutation, i.e. that the kinase domain is simply a nonfunctional tail to what is, in effect, a DCX variant, is eliminated by the findings that recombinant DCK2 expressed in bacteria or mammalian cells, is competent to both autophosphorylate and phosphorylate exogenous substrates (Figs. 5 and 6). This ability is independent of the DC domain as removal of the latter was without effect on the peptide kinase activity of DCK2 (Fig. 5D).

Nonetheless, although the DC and kinase domains are capable of independent activities, their evolutionary conservation in active forms in a single molecule suggests that they must co-participate in whatever physiological function may be effected by DCK2. This hypothesis is supported by the observation that both the DC and kinase domains of ZYG-8 are important for spindle positioning during cell division in C. elegans embryos (22). Two potential mechanisms that might account for the presence of both domains in DCK2 appear plausible. One may be referred to as the “targeting” model. In this mechanism the DC domain functions as an interaction module to position the kinase for efficient substrate phosphorylation. Data showing DCK2 phosphorylation of an HMW-MAP is compatible with this mechanism (Fig. 5B). In this model, the DC domain through its ability to bind to MTs acts to enhance the efficacy of the kinase domain. Another possibility is a “phosphorylation switch” mechanism. In this scheme, the kinase domain, through its ability to catalyze DCK2 autophosphorylation and thereby control the MT binding affinity of the DC domain, acts to reversibly regulate (in concert with a cellular phosphatase) MT dynamic instability. In contrast to the targeting model, here the kinase domain acts to enhance the functional capability of the DC domain. The data of Fig. 6 showing reversible regulation of DCK2-MT interaction by phosphorylation/dephosphorylation is consistent with this model. Because neither the ability to phosphorylate MAPs nor regulate its MT binding by autophosphorylation has been reported for DCK1 whether either of these two mechanisms are operative for the latter is unknown at present.

It will be of interest to map the autophosphorylation sites of DCK2. Given the number of patient mutations in DCX that influence MT binding, it would not be surprising if one or more DCK2 autophosphorylation sites are located in its DC domain or associated SP domain. The advantage to the cell of the phosphorylation switch mechanism is that in response to signaling serving to activate DCK2, MT dynamic instability could be reversibly regulated by a DCK2 autophosphorylation/dephosphorylation cycle without the need for additional components. The current data do not, however, eliminate the possibility that autophosphorylation in vitro mimics phosphorylation in vivo by a heterologous kinase, a possibility that needs to be addressed in future studies. It is also interesting to speculate that if the MT binding ability of DCX is similarly regulated by phosphorylation, evolutionary removal of an associated kinase domain could serve to create new signaling possibilities through the necessary involvement of heterologous kinase(s). In this context, recent results have documented changes in MT binding of DCX upon its phosphorylation by cAMP-dependent protein kinase, MARK, and Cdk5 (45, 46).

It is clear that the current data do not definitively validate either the targeting or phosphorylation switch models. Indeed, it is possible that both may participate in “hybrid” mechanisms. For example, in the targeting model, the capacity of DCK2 to stabilize MTs might aid in the assembly of a multicomponent protein complex that could serve as a scaffold for co-localizing DCK2 and its substrates. Conversely, in the phosphorylation switch mechanism, MT-bound DCK2, responding to a signal resulting in its activation, might both autophosphorylate and phosphorylate an associated MAP(s), with dissociation of both, synergistically enhancing MT dynamic instability.

Whatever the mechanism, several lines of evidence suggest that DCK2 is not physiologically redundant with DCX. First, although DCK2 is expressed within the same time window of embryonic cerebrocortical development as DCX (Fig. 7A), to date no patient mutations in any of the known forms of lissencephaly have been mapped to DCK2 (this is true for DCK1 as well). It is always possible that additional mapping data may help establish linkage to another neurological disorder, but examination of the cytogenetic locus in the immediate vicinity of the DCK2 gene (4q31.23) reveals no obvious candidates. It is also the case that disruption of cortical lamination due to missense DCX mutations is not suppressed by the (presumably) normal expression of DCK2 and/or DCK1. Second, the lack of down-regulation of DCK2 postnatally in comparison to DCX (Fig. 7B) and, potentially, to DCK1-α (13, 18, 39) suggests that unlike the latter, DCK2 may play an important role in cytoskeletal remodeling events in the nervous system post-natally.

DCK2 is targeted to terminal segments of axons and dendrites (Fig. 8). DCK1 has also been reported to be present in the tips of neurites, although whether it is found in both types of processes has not been established (43). Taken together, the neuronal localization and temporal expression of DCK2 are consistent with its potential involvement in a range of important neuronal events such as process extension and growth cone motility, synaptogenesis, or synaptic remodeling as a consequence of neuronal activity (47–49). Ultimately, the development of mouse knock-out or transgenic models may be necessary to precisely define precisely the physiological role of DCK2.

In conclusion, expansion of the DC gene family through the identification of DCK2 supports the idea that the DC sequence plays a critical role in transducing extracellular cues and their intracellular signals into changes in MT dynamics. In particular, based on an ability to interact with MTs in a fashion regulatable by phosphorylation and to localize to terminal segments of axons and dendrites, regions in which MTs are dynamically unstable, DCK2 should be considered a potential candidate mediator of the rapid cytoskeletal rearrangements that occur in response to neuronal signaling events (50). Details of such events and of their underlying signal transduction pathways remain to be established through future investigations.

Acknowledgments—We thank Bob Hard, Joe Gleeson, Joan Baizer, Limin Shang, Hui Qin, and Gene Giossierek for stimulating discussions during the course of these studies.

Note Added in Proof—Recently, DCX phosphorylation by c-Jun N-terminal kinase (JNK) has also been demonstrated (Gdalyahu, A., Ghosh, I., Levy, T., Sapir, T., Sapoznik, S., Fishler, Y., Azoulai, D., and Reiner, O. (2004) EMBO J. 23, 823–832).
Doublecortin Kinase-2, a Novel Doublecortin-related Protein Kinase Associated with Terminal Segments of Axons and Dendrites
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*J. Biol. Chem.* 2005, 280:8531-8543.
doi: 10.1074/jbc.M411027200 originally published online December 20, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M411027200

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