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# Ndel1 Controls the Dynein-mediated Transport of Vimentin during Neurite Outgrowth*[S]

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**Ndel1, the mammalian homologue of the *Aspergillus nidulans* NudE, is emergently viewed as an integrator of the cytoskeleton. By regulating the dynamics of microtubules and assembly of neuronal intermediate filaments (IFs), Ndel1 promotes neurite outgrowth, neuronal migration, and cell integrity (1–6). To further understand the roles of Ndel1 in cytoskeletal dynamics, we performed a tandem affinity purification of Ndel1-interacting proteins. We isolated a novel Ndel1 molecular complex composed of the IF vimentin, the molecular motor dynein, the lissencephaly protein Lis1, and the cis-Golgi-associated protein αCOP. Ndel1 promotes the interaction between Lis1, αCOP, and the vimentin-dynein complex. The functional result of this complex is activation of dynein-mediated transport of vimentin. A loss of Ndel1 functions by RNA interference fails to incorporate Lis1/αCOP in the complex, reduces the transport of vimentin, and culminates in IF accumulations and altered neuritogenesis. Our findings reveal a novel regulatory mechanism of vimentin transport during neurite extension that may have implications in diseases featuring transport/trafficking defects and impaired regeneration.**

The cytoskeleton constitutes a highly organized structure formed by the interconnection of three filamentous networks (microtubules (MTs),[3] intermediate filaments (IFs), and microfilament) and their associated proteins (7–10). The dynamics of these networks regulate essential intracellular functions such as transport and trafficking and, therefore, impact on cell division, morphology, and integrity (7–10).

Named according to their intermediate diameter (10 nm), IFs constitute the largest family of cytoskeletal proteins. Up to 60 genes encoding for IFs are expressed in a tissue-specific and spatio-temporal manner in mammals (7, 11, 12). After synthesis in the cytoplasm, a fraction of soluble IF proteins is rapidly incorporated into the polymeric filamentous network, referred to as the "insoluble" fraction (7, 12). In parallel, individual IF subunits, dimers, or small oligomers that constitute the "soluble" fraction translocate rapidly along MTs via molecular motors in structures termed particles (13, 14). This model is particularly relevant for the assembly of the IF vimentin (15, 16) and more controversial in the case of neuronal IFs (NFs) (17–19).

The neurofilament proteins (NF-L, NF-M, and NF-H) are the most abundant IFs in mature central nervous system neurons (12). We recently demonstrated that Ndel1, the mammalian homologue of the *Aspergillus nidulans* NudE, a protein implicated in nuclear distribution during hyphal growth, binds directly to the key subunit NF-L, thereby regulating NF assembly (5). Ndel1 also interacts with the Disrupted-in-Schizophrenia protein 1 (DISC1) to regulate MT dynamics during neurite outgrowth in neuroblastoma PC-12 cells and during neuronal migration in the developing cortex. Interestingly, Ndel1 associates with the molecular motors dynein/dynactin (20–22), and numerous independent reports documented the dynein transport of the vimentin, an IF important for glial activation and neurite formation and exhibiting pro- and anti-regenerative properties following axonal injury (13–16, 23–29). Whether Ndel1 associates with the dynein-vimentin complex during neurite outgrowth and regulates vimentin dynamics remains elusive.

Using a proteomic approach of tandem affinity purification, we now identify Ndel1 as a key component in the vimentin-dynein complex in cells and discover that this complex is formed during neurite extension. Nde11 participates in the formation of a quint-partite complex by promoting interaction between the lissencephaly protein Lis1, the vesicular protein αCOP, and the vimentin-dynein complex. The results of this complex are activation of dynein-mediated transport of vimentin and functional consequences on neurite outgrowth.

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[3] The abbreviations used are: MT, microtubule; IF, intermediate filament; NF, neuronal IF; DISC1, Disrupted-in-Schizophrenia protein 1; GFP, green fluorescent protein; PBS, phosphate-buffered saline; RNAi, RNA interference; DIC, dynein intermediate; DHC, dynein heavy chain; COP, coat protein; RFP, red fluorescent protein.

## EXPERIMENTAL PROCEDURES

*Ndel1 Complex Purification*—The purification procedure was performed as previously described (30). Briefly, recombinant retroviruses expressing a bicistronic messenger RNA containing open reading frames of FLAG-HA-tagged human Ndel1 and interleukin-2 receptor-$\alpha$ were constructed and transduced into suspension HeLa cells. The infected HeLa cells were selected twice using anti-interleukin-2 receptor monoclonal antibody-conjugated magnetic beads, and the resulting FLAG-HA-Ndel1-stable cell lines were grown in suspension. Soluble extract was made from 40 liters of cells. The Ndel1 complex was tandem-purified in detergent-free buffers by using anti-FLAG M2 monoclonal antibody-conjugated agarose beads (Sigma) and then anti-HA 12CA5 monoclonal antibody-conjugated beads. FLAG-HA double purified material was separated by 4–20% gradient SDS-PAGE and stained with Coomassie Blue. The various protein bands were excised and analyzed by mass spectrometry at the Harvard Medical School Taplin Biological Mass Spectrometry Facility.

*Western Blots and Immunoprecipitations*—Total protein extracts of CAD cells were obtained by homogenization in SDS-urea $\beta$-mercaptoethanol (0.5% SDS, 8 M urea in 7.4 phosphate buffer). Soluble fractions were prepared in Triton X-100 (10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA (pH 8.0), and 1% Triton), HEPES (50 mM HEPES, pH 7.0, 150 mM NaCl, 1 mM EDTA, 0.1% Nonidet P-40, 25 mM NaF, 10 mM $Na_3VO_4$ and 1 mM dithiothreitol) or E1A lysis buffer (50 mM Tris-HCl (pH 7.5), 250 mM NaCl, 5 mM EDTA (pH 8.0), and 0.1% Nonidet P-40) with a mixture of protease inhibitors (leupeptin, pepstatin, and aprotinin). The protein concentration was estimated by the Bradford procedure (Bio-Rad). Proteins (20–50 $\mu$g) were fractionated on 7.5% SDS-PAGE and blotted onto a nitrocellulose or polyvinylidene difluoride membrane for Western blot analysis. Membranes were incubated with antibodies against Ndel1 (210 and 211) (3, 5), Lis1 (486) (21) dynein intermediate chain (MAB 1618, Chemicon), actin (C4, MAB1501, Chemicon), vimentin (Abcam, Chemicon), $\alpha$COP I (Abcam), p58 (Sigma-Aldrich), FAK (N-20, Santa Cruz Biotechnology), GFP (Molecular Probes), acetylated tubulin (Abcam), tyrosinated tubulin (Abcam), GAP-43 (Abcam), and DISC1 (a kind gift from Dr. Brandon, Wyeth). The Western blots were revealed by chemiluminescence (Renaissance, Western blot kit, PerkinElmer Life Sciences). Quantifications were corrected with levels of actin or $\alpha$-tubulin and performed with the Labscan program (Image Master, two-dimensional software, v. 3.10, Amersham Biosciences).

*Yeast Two-hybrid Interactions*—Cloning of human cDNA for full-length (amino acid residues 1–345) of Ndel1 in pPC97 vector (pDBLeu, Invitrogen) for expression as a GAL4 DNA binding domain fusion protein was described previously (5, 21). pPC86-NFL rod construct for expression of NFL rod domain (77–364) as a GAL4 activation domain was a kind gift from Drs. Michael Ehlers (Duke University) and Richard Huganir (Johns Hopkins University). pPC86-vimentin, pPC$\alpha$-COP, and pPC86-DISC1 fragment constructs for expression as a GAL4 activation domain was a kind gift from Dr. Wallace Ip (University of Cincinnati), Blanche Schwappach (University of Heidelberg), and Dr. Akira Sawa (Johns Hopkins University), respectively. Yeast two-hybrid assays were performed as described previously (5).

*Cell Culture, Immunofluorescence, and Live Imaging*—Cultures of CAD cells were performed as described previously (3, 5). Following transfection with Lipofectamine 2000 (Invitrogen) in serum-free medium, CAD cells stop proliferating and exhibit morphological characteristics and molecular signatures of primary neurons such as expression of class III $\beta$-tubulin and enzymatically active tyrosine hydroxylase (31). After transfection, CAD cells were washed once in 37°c PBS and then fixed with 4% paraformaldehyde/PBS for 10 min at room temperature. After fixation, the cells were washed twice with PBS and then blocked with PBS, 5% skimmed milk (or 3% bovine serum albumin), 0.1% Triton X-100 for 1 h. After fixation, cells were incubated with primary antibody (vimentin, Ndel1, Tubulin) diluted in the blocking buffer for 1 h at 37°c or overnight in a cold room. Following three washes with PBS, secondary antibodies (Alexa 488, 594, Cy2, Cy3) diluted in blocking buffer were added to the cells for 1 h at 37 °C or 2 h at 25 °C. The cells were finally washed three times with PBS followed by a wash with water before mounting with Immuno-Mount. Pictures were taken with a C-1 eclipse Nikon confocal microscope.

For live imaging, 24 h after plating, CAD cells were first transfected with a construct encoding a random sequence without homology to any known mRNA (control) or Ndel1 RNAi, and an RFP-centrin construct to mark the cells that have integrated the control or Ndel1 RNAi (see supplemental Fig. S2). After 24 h, when Ndel1 RNAi efficiently knocks down the levels of Ndel1 protein (3, 5), the same cells were transfected a second time with a GFP-vimentin construct. 6–8 h after the second transfection, live imaging of GFP-vimentin dots (a pattern distinct of the soluble pool of IF subunits) was performed. A picture was taken every 30 s for a total of 10 min per cell; up to 56 cells were recorded during four independent experiments for a total of 190 vimentin dots. Note that beyond 10 h of post-transfection, the GFP-vimentin subunits were readily found in filamentous form (supplemental Fig. S2). To quantify the movements of GFP-vimentin, only uni-nuclear cells that expressed one RFP-centrin dot (one centrosome), had Ndel1 depletion, and expressed mobile GFP-vimentin dots (non filamentous) were taken. Clear and distinct moving vimentin dots were picked randomly and far apart from any GFP-vimentin aggregates to avoid eventual blockage of transport. Dots with no lateral, ascendant, and descendant movements were considered immobile.

*Generation, Characterization of siRNA Sequences, and RNAi Vectors*—RNAi sequences were selected based on the criteria proposed previously (32). Complementary hairpin sequences or oligonucleotides were commercially synthesized and cloned into pSilencer 2.0 under promoter U6 (Ambion). The sequence for Ndel1 comprises bp 276–294 (GCAGGTCTCAGTGTTAGAA) (3, 5). A random sequence without homology to any known mRNA was used for control RNAi. All RNAi constructs were tested in 3T3, COS-7, and CAD cells by both Western blot and immunofluorescent staining.

*Sucrose and Glycerol Gradients*—High speed supernatants were made from spinal cord and nerve homogenates of

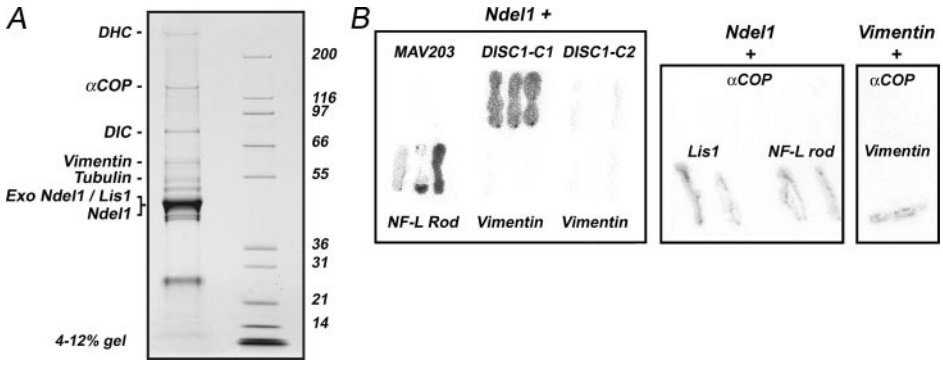


FIGURE 1. **Ndel1 tandem affinity purification and yeast two-hybrid interactions.** *A*, tandem affinity purification of Ndel1-interacting partners isolated the IF vimentin, αCOP of COP I, dynein heavy and intermediate chains (*DHC* and *DIC*), Lis1, and exogenous/endogenous Ndel1. *B*, yeast two-hybrid analysis revealed direct association between Ndel1 and Lis1 and absence of direct interaction between Ndel1 and vimentin, Ndel1 and αCOP, and αCOP and vimentin. Positive controls for Ndel1 interaction: NF-L rod domain and DISC1-C1 fragment (4, 5); positive control for vimentin: vimentin; negative controls: untransformed strains or strains co-transformed with Ndel1 and DISC1-C2 fragment (4).

4-month-old wild-type littermates or cell lysates in buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA) or a modified version of radioimmune precipitation assay buffer without detergents plus protease inhibitors and fractionated on sucrose gradients. 200 μl of homogenates was overlaid onto a 12-ml, 5–20% sucrose gradient and centrifuged at 32,000 × *g* for 18 h at 4 °C in TiSW41 swing buckets in a Beckman L8–70M Ultracentrifuge. Sucrose gradients were previously made in Beckman Ultra-Clear tubes using a gradient maker Hoeffer 15 as described by the manufacturer's protocol (Amersham Biosciences). During all centrifugations, gradients loaded with standards (1 mg) such as bovine serum albumin, catalase, or thyroglobulin were processed simultaneously. Following centrifugation, 12 fractions of 1 ml were collected for each sample. Glycerol gradients were performed according to a previous study (3).

## RESULTS

*Novel Ndel1 Interacting Partners in Cells*—A proteomic-based tandem affinity purification for Ndel1 was employed to determine fine molecular complexes involved in cytoskeletal dynamics and rearrangement. Briefly, Ndel1 was tagged at the N terminus with two affinity tags (FLAG and HA epitopes) and stably expressed in HeLa cells in suspension. Exogenous Ndel1 was expressed at a physiological level, *i.e.* 1.2-fold the level of endogenous Ndel1 (data not shown). Following several detergent-free fractionations and co-immunoprecipitations (see "Experimental Procedures" and Ref. 30), the soluble cytoplasmic fraction was isolated and Ndel1 interacting proteins were identified by mass spectrometry. From the screen, some of the proteins recovered were: endogenous Ndel1, dynein intermediate (DIC), and heavy chains (DHC), the lissencephaly gene product, Lis1, the IF vimentin, and the trafficking-associated protein αCOP (Fig. 1*A*). In agreement with previous studies (3, 21, 22, 33, 34), our results indicated that Ndel1 can form a complex with itself, DIC, DHC, and Lis1. Remarkably, we also uncovered that Ndel1 is present in complexes with vimentin, an IF important for neurite extension with pro- and anti-regeneration properties (7, 23, 24, 26, 28, 29, 35, 36), as well as with αCOP, a subunit of the non-clathrin-coated vesicular coat proteins (COPs) coatomer COP I, which is essential for the retrieval and transport of vesicles from the cis-Golgi back to the endoplasmic reticulum (37, 38). Because the soluble fraction was isolated, the IFs obtained were likely in a non-filamentous form, and αCOP was not membrane-associated. It is noteworthy that, under our experimental conditions, Erk and importins, which have been shown to associate with dynein and vimentin (26), were not found in the tandem affinity purification. Neither were other Ndel1-interacting partners such as Fez-1 and DISC1 (1, 2, 39, 40).

*Ndel1 Interacts Directly with Lis1 but Not with vimentin and αCOP in the Yeast Two-hybrid System*—To further understand the interactions between members of the complex, we used the yeast two-hybrid system. In the assay, the association between Ndel1 and Lis1 is direct while the interactions between Ndel1 and vimentin, Ndel1 and αCOP, and αCOP and vimentin were not direct (Fig. 1*B*). Neurofilament light chain (NF-L) and a Ndel1-interacting fragment of DISC1 (c1), which have been reported to interact directly with Ndel1 in the same system (4, 5) (Fig. 1*B*), were used as positive controls. Because Lis1 is known to interact directly with dynein (3,20–22,41), which transports vimentin cargo (16, 26), Ndel1 may associate with vimentin via Lis1/dynein. It is noteworthy that we cannot rule out the possibility that Ndel1 and vimentin, Ndel1 and αCOP, and αCOP and vimentin may interact directly in other systems.

*Formation of the Novel Ndel1 Complex in Neuronal Cells Extending Neurites*—To validate the existence of the quint-partite complex Ndel1-vimentin-dynein-Lis1-αCOP in cells, we performed additional co-immunoprecipitations on soluble lysates of differentiating neuroblastoma CAD cells (see "Experimental Procedures") transfected with a random RNAi sequence (used as a control) and undergoing neurite outgrowth. Lis1 and αCOP co-immunoprecipitated with Ndel1 in differentiating CAD cells (Fig. 2*A*). Furthermore, vimentin, Lis1, Ndel1, and αCOP also co-immunoprecipitated with DIC (Fig. 2*B*). Finally, Ndel1, Lis1, and vimentin co-immunoprecipitated with αCOP (Fig. 2*C*). These results confirmed the existence of the quint-partite complex in neuronal cells undergoing neurite outgrowth.

To define the role of Ndel1 in the formation of the complex during neurite outgrowth, we knocked down the levels of Ndel1 protein in CAD cells extending neurite using a specific RNAi sequence (3, 5) and assessed the interactions between vimentin, dynein, Lis1, and αCOP. Importantly, in cells depleted of Ndel1, αCOP and Lis1 did not co-immunoprecipitate with DIC and vimentin using DIC antibodies for immunoprecipitations (Fig. 2, *B* and *D*). In the same cells lacking Ndel1, vimentin did not co-immunoprecipitate with αCOP and Lis1 (Fig. 2, *C* and *D*). These co-immunoprecipitation experiments suggested that

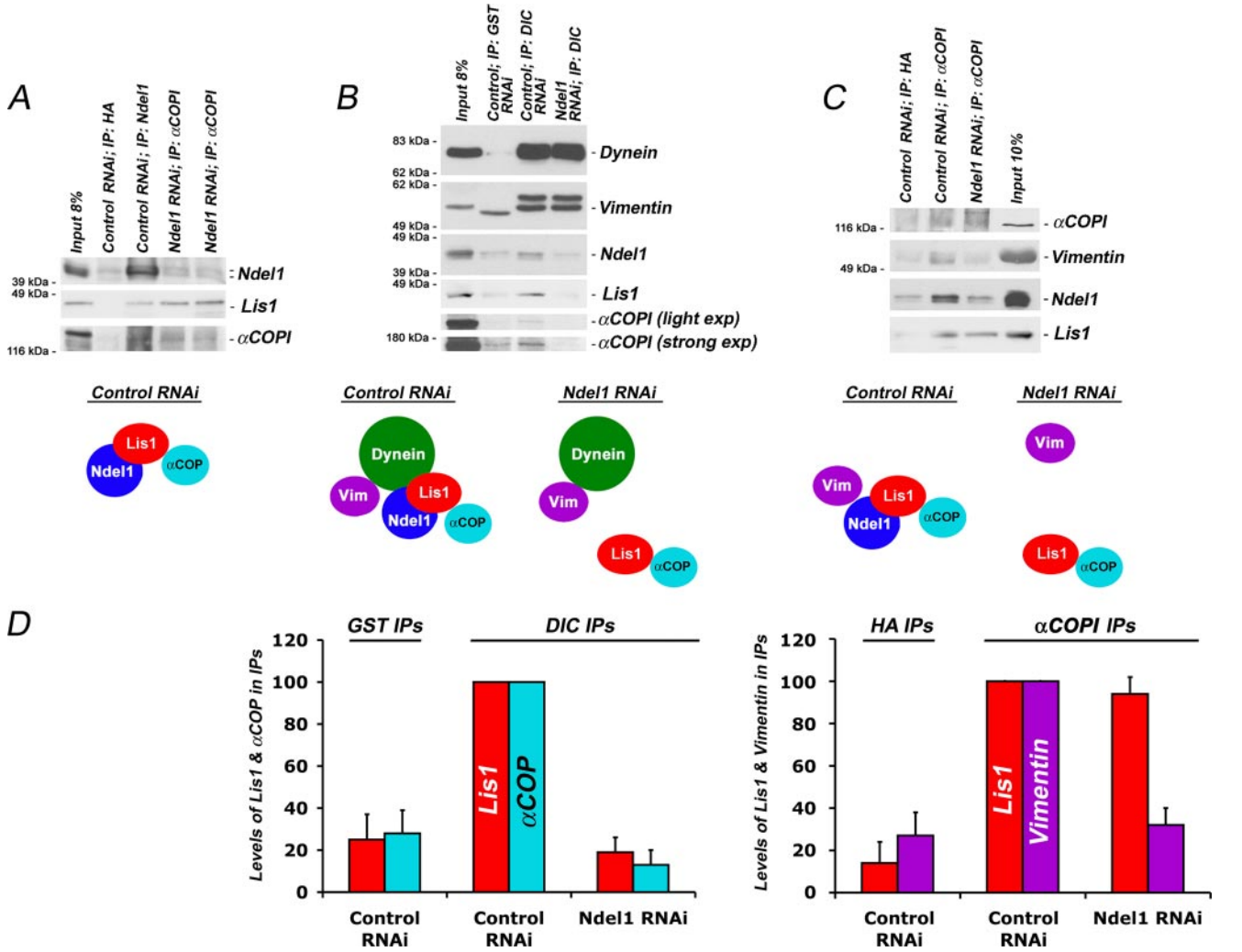


FIGURE 2. **Molecular assembly of the quint-partite complex during neurite outgrowth.** *A*, αCOP I co-immunoprecipitated with Ndel1 and Lis1 in CAD cells transfected with a scramble RNAi vector (control cells). Ndel1 RNAi knocked down efficiently Ndel1 protein levels in CAD cells (+ see Fig. 3*B*). *B*, Lis1 and αCOP co-immunoprecipitated with DIC and vimentin in control cells but not in cells depleted of Ndel1. The high molecular band of vimentin might be a post-translated form of vimentin. *C*, vimentin co-immunoprecipitated with Lis1 and αCOP in control cells but not in cells depleted of Ndel1. *D*, quantifications of the amount of Lis1, αCOP, and vimentin in immunoprecipitates obtained from soluble lysates of control or Ndel1 RNAi-treated cells with glutathione *S*-transferase, HA (controls), or DIC antibodies. Four independent experiments in triplicate were analyzed for each condition (control and Ndel1 RNAi). *DIC/DHC*, dynein intermediate/heavy chain. *IP*, refers to the antibodies used for immunoprecipitations.

Ndel1 carries Lis1/αCOP to the vimentin-dynein complex in neuronal cells. Consistent with this view, a larger pool of Lis1 did not co-fractionate with dynein in glycerol gradients made from CAD cells depleted of Ndel1 (supplemental Fig. S1). The loss of Ndel1 also mislocalized up to 25% of total αCOP as detected by both glycerol and sucrose gradients (supplemental Fig. S1). Together, these results indicated that 1) Ndel1 participates in the formation of the quint-partite complex by promoting the interaction between Lis1/αCOP and vimentin-dynein complexes in neuronal cells undergoing neurite outgrowth and 2) the association between Ndel1 and vimentin is likely mediated through Lis1 and dynein.

*Ndel1 Controls the Transport of Vimentin Cargo during Neurite Extension*—In association with the Disrupted-in-Schizophrenia protein 1 DISC1, Ndel1 has been shown to regulate neurite outgrowth of PC-12 cells via MT stability and dynamics (1, 2, 4). In agreement with these studies, we found that depletion of Ndel1 protein by RNAi altered neurite extension in neuroblastoma CAD cells (Figs. 3*A* and 4*B*). These cells lacking Ndel1 did not display the typical polar morphology of neighboring untransfected cells and cells transfected with a control RNAi, characterized by dynamic neurite extension and elongated cell body (Figs. 3*A* and 4*A*, *red* and *orange arrows*). Interestingly, the defect in neurite outgrowth was not due to loss of MTs, because MT structures and levels of tyrosinated (unpolymerized) tubulin in these cells were similar to control cells (Fig. 3, *A–C*). Levels of acetylated tubulin, a marker for stable MTs, were also equal in control and Ndel1 RNAi-treated cells (Fig. 3, *B* and *C*). Furthermore, levels of DISC1, an Ndel1-interacting protein and co-regulator of MT stability, were unchanged in Ndel1 RNAi-treated cells (Fig. 3, *B* and *C*). These results suggested that Ndel1 contributes to neurite outgrowth by an alternative mechanism that does not involve DISC1-dependent MT stabilization.

Based on our biochemical data, we hypothesized that Ndel1 affects vimentin, which is critical for neurite extension in neu-

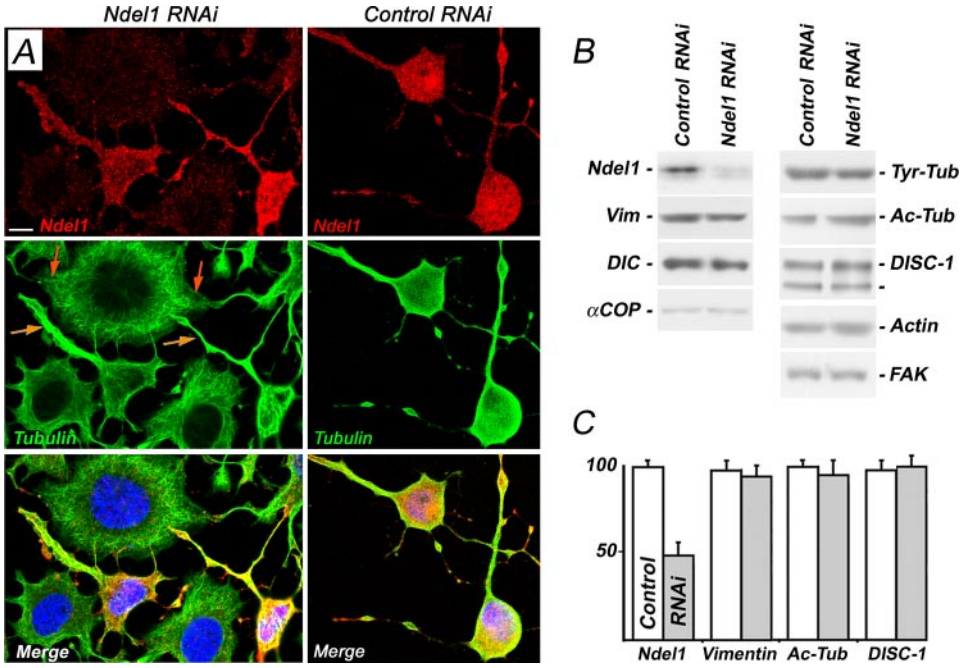


FIGURE 3. **Neurite extension defects in Ndel1 RNAi-depleted CAD cells independent of DISC1-mediated MT stabilization.** *A*, neurite outgrowth defects in Ndel1-RNAi treated CAD cells are not caused by MT destabilization as detected with confocal microscopy images. Note the normal formation of MT network in the Ndel1 RNAi-treated cells. CAD cells depleted of Ndel1 protein remain undifferentiated (*red arrows* pointing to immature neuronal protrusions similar to the ones in Fig. 4*B*). Cells with no down-regulation of Ndel1 or cells transfected with control RNAi nicely extended processes (*orange arrows*). *Red*, Ndel1, Cy3; *green*, tubulin, Cy2; *blue*, 4′,6-diamidino-2-phenylindole. *Bar*, 10 μm. *B*, Western blots depicting the unchanged levels of vimentin, αCOP, DIC, DISC1, tyrosinated (Tyr) tubulin, and acetylated (stable) tubulin in Ndel1 RNAi-treated cells. Actin and FAK were used as loading controls. *C*, quantifications of the unchanged protein levels in *B*. Four independent experiments in triplicate were analyzed for each protein.

roblastoma cells and primary cultured neurons (23, 24, 27). Indeed, morphologically, Ndel1-depleted CAD cells mimicked the outgrowth defects observed in neurons and neuroblastoma cell lines lacking vimentin (23, 24, 27). However, we found no difference in levels of vimentin in Ndel1-RNAi-treated cells when compared with control cells (Fig. 3, *B* and *C*). Because Ndel1 facilitates the interaction between Lis1 and the vimentin-dynein complex (Fig. 2), and Lis1 can activate the ATPase motor of dynein (3, 34, 41, 42) to transport cargoes such as vimentin, we suspected that Ndel1 might control vimentin dynamics during neurite outgrowth. We then assessed the motility of the dynein-dependent vimentin cargo in differentiated CAD cells expressing GFP-vimentin but depleted of Ndel1. The procedures using multiple transfections and live imaging are described in the "Experimental Procedures" and supplemental Fig. S2.

To quantify the movements of GFP-vimentin, we randomly took mononuclear cells that had a depletion in Ndel1 protein, expressed one RFP-Centrin dot (a centrosomal marker co-transfected with the Ndel1 RNAi) and GFP-vimentin dots (non filamentous subunits) (supplemental Fig. S2). A representative Western blot in supplemental Fig. S2 depicts the proteins that have been expressed and knocked down. In control cells ($n = 21$), the average speed of the GFP-vimentin moving dots selected randomly ($n = 95$) was evaluated at 0.083 ± 0.017 μm/s (see supplemental Control RNAi movie and Fig. 4*A*). Remarkably, in Ndel1 RNAi-treated cells ($n = 35$ for 95 dots), the average speed was reduced by half (0.037 ± 0.014 μm/s) ($P$ ($T < t$) two tails: <0.001) (see supplemental Ndel1 RNAi movie and Fig. 4*B*). It is noteworthy that the speed of dynein-GFP-vimentin in 54–56 h plated double-transfected CAD cells is almost 10 times slower than the GFP-vimentin motility in BHK-21-spreading cells, 30–45 min after trypsinization/replating (15). Indeed, cytoskeletal remodeling and transport appear much more dynamic in freshly plated cells.

When the number of vimentin dots is plotted as a function of rate, control cells exhibited a typical Gaussian distribution (Fig. 5), which is consistent with previous studies on vimentin dynamics (15). In contrast, Ndel1-RNAi-treated cells did not exhibit such classic Gaussian distribution. Indeed, an increased number of GFP-vimentin dots with low speed and a decreased number of GFP-vimentin dots with high speed were found (Fig. 5). Of note, the higher number of stationary GFP-vimentin dots in Ndel1 RNAi-treated cells *versus* control cells was not included in the calculation of the speed.

The altered vimentin dynamics in Ndel1 RNAi-treated cells was further highlighted by the high number of immobile dots in these cells. In control cells ($n = 21$), only 10.3% ± 3.9% of the total GFP-vimentin dots ($n = 646$) are immobile. In the Ndel1 RNAi-treated cells ($n = 35$), up to 52.5% ± 11.3% of the dots ($n = 612$) did not move. These immobile dots often accumulated in cell bodies and immature protrusions of RNAi-treated CAD (Fig. 4*B*, *colored boxes* and *red arrows*) in a manner reminiscent of IF accumulations in neurodegenerative disorders featuring transport defects (see supplemental Ndel1 RNAi movie). This is in striking contrast to control cells that had GFP-tagged vimentin particles moving bi-directionally in the cell body and neurites (Fig. 4*A*, *colored boxes* and *arrows*) (see Control RNAi movie). This movement can be visualized at different times by the distinct pattern, distribution, and intensity of the GFP-vimentin dots (Fig. 4 and supplemental movies).

Based on the lack of involvement of DISC1-dependent MT stabilization, the importance of vimentin dynamics for vimentin assembly and functions, we propose that defects in neurite outgrowth of Ndel1 RNAi-depleted cells are caused by alteration in vimentin dynamics. By favoring the interaction between Lis1 and dynein-vimentin, Ndel1 controls the ability of dynein to move vimentin cargo, thereby impacting neuritogenesis (Fig. 6).

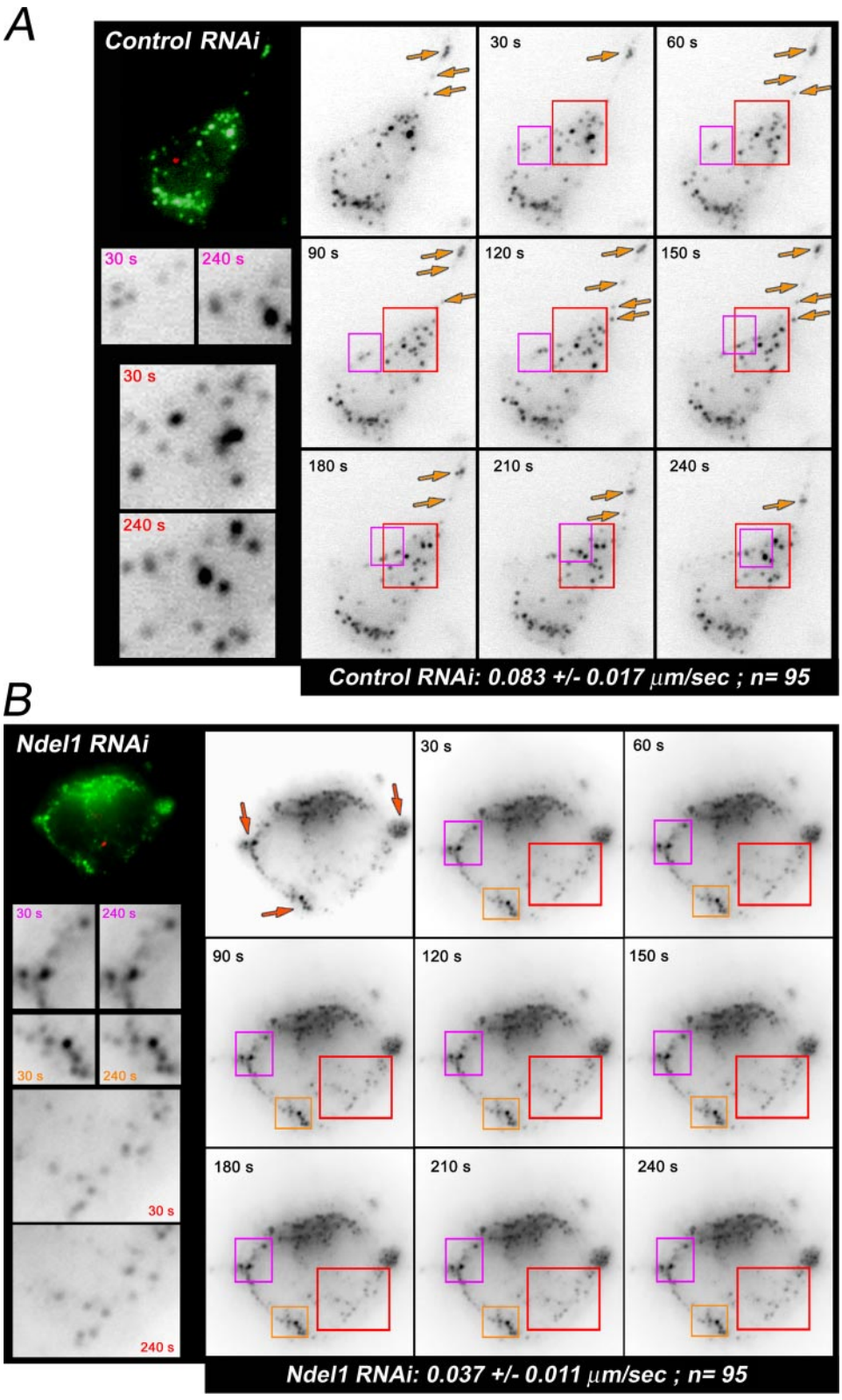


FIGURE 4. **Vimentin dynamics in the presence or absence of Ndel1 in CAD cells.** Photographs taken every 30 s (s) depicting the movements of GFP-vimentin dots in a CAD cell triply-transfected with RNAi scramble/RFP centrin (*red*) and GFP-vimentin (*A*) or Ndel1 RNAi/RFP centrin and GFP-vimentin (*B*). The average speed of the GFP-vimentin dots ($n = 95$) in Ndel1 RNAi-treated cells ($n = 35$) was half the speed of GFP-vimentin dots ($n = 95$) in scramble RNAi-treated cells (control, $n = 21$ cells; 95 dots) ($p$ ($T < t$) two tails: $<0.001$). *Orange arrows* point to vimentin particles translocating in neuronal processes. *Red arrows* point to vimentin particles accumulating at immature protrusions. Note that immobile dots were not included in the calculation (see main text for details). *Red*, *orange*, and *pink boxes* frame GFP-vimentin dot movements over 4 min. *Frames* of the *boxes* that were enlarged at 30 and 240 s highlight the changes or absence of changes of pattern, indicative of mobility or immobility of GFP-vimentin, respectively. For complete sequence, see supplemental Control and RNAi movies.

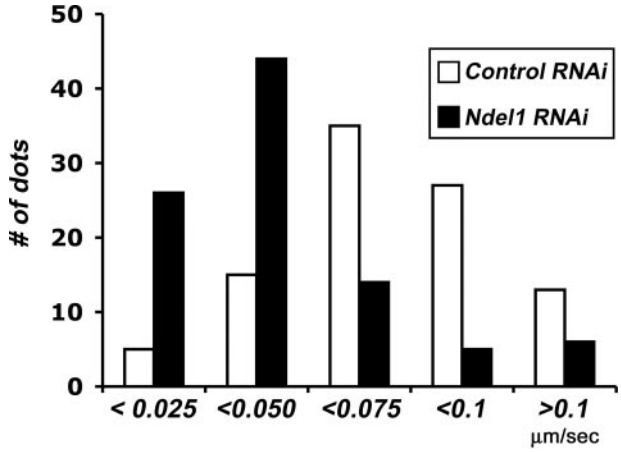


FIGURE 5. **Graph analysis of the vimentin dynamics.** Gaussian distribution of graph depicting the number of dots *versus* speed derived from Control RNAi-treated cells. Following depletion of Ndel1 protein with RNAi, the vimentin dynamics are altered and the Gaussian distribution is lost (Control RNAi-treated cells ($n$ = 21; 95 dots); Ndel1 RNAi-treated cells ($n$ = 35; 95 dots)).

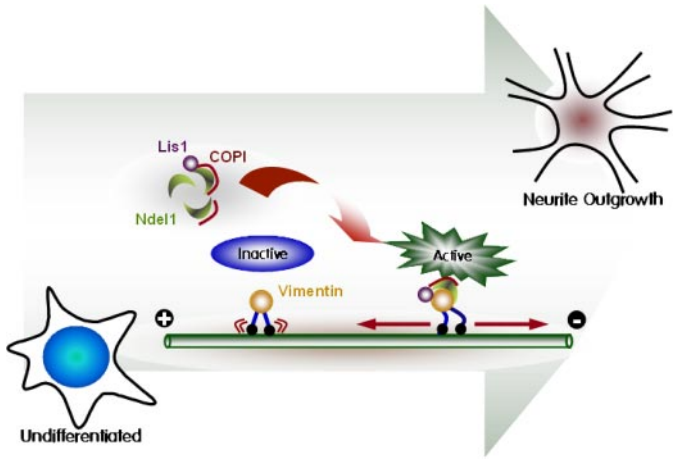


FIGURE 6. **Formation of the Ndel1 quint-partite complex during neurite extension.** Ndel1 carries Lis1 and αCOP to an inactive vimentin-dynein complex. The formation of the quint-partite complex activates the transport of vimentin by dynein during neurite outgrowth and differentiation of CAD cells.

## DISCUSSION

*A Novel Ndel1 Molecular Complex during Neurite Outgrowth*—With over a dozen cytoskeletal binding partners, Ndel1 is emergently viewed as an integrator of the cytoskeleton in mitotic and post-mitotic cells (3–5, 20–22, 40, 43–45). In this study, we uncovered the formation of a novel Ndel1 molecular complex composed of vimentin, dynein subunits, Lis1, and αCOP (Figs. 1 and 2). We showed that Ndel1 controls the activity of dynein to move vimentin cargo in neuroblastoma CAD cells (Figs. 4 and 5). Indeed, depletion of Ndel1 by RNAi impairs vimentin dynamics (which is critical for vimentin assembly and functions), resulting in neurite outgrowth defects reminiscent of those exhibited by cells depleted of vimentin (23, 24, 27). Previously, Ndel1 together with DISC1 have been shown to be important for neurite outgrowth in PC-12 cells (2). However, in our Ndel1 RNAi-treated CAD cells, the MT network and levels of DISC1 are unperturbed. The different cell type may account for the difference in mechanisms.

In our model, Ndel1 likely controls vimentin transport via its direct association with dynein/dynactin molecular motors (Fig. 1). Indeed, Ndel1 binds the P1 loop ATPase domain of DHC, which is essential for dynein activity (20, 34, 41, 42). The novel function of Ndel1 on vimentin dynamics is distinct from its role on polymerization of NFs via direct interaction and stabilization of NF-L in mature neurons (5). This mechanism is likely to be critical for IF transport over long distances, turnover of the IF network, and cytoskeletal remodeling in diseased conditions. Finally, recent studies indicate that Ndel1 is in complex with Kinesin motors suggesting that it might coordinate the activity of both retrograde and anterograde motors (46, 47). Thus, the altered vimentin dynamics observed in the Ndel1 RNAi-treated cells might be a combination of both retrograde and anterograde movement defects.

*Putative Functions of αCOP in the Complex*—Unexpectedly, we found the presence of αCOP in the Ndel1-dynein-vimentin-Lis1 complex, a protein essential for the retrieving of vesicles at the cis-Golgi back to the endoplasmic reticulum. We found that the non-membrane-associated form of αCOP is carried by Ndel1 to the vimentin-dynein complex (Figs. 1 and 2). COP II proteins that bud ER-derived anterograde vesicles have been solely used as a marker for the dynein/dynactin activity (6, 33). Thus, the interaction between Ndel1 and αCOP of COP I (but not COP II) and its presence in the Ndel1-dynein-vimentin-Lis1 complex are novel. In the quint-partite complex, αCOP may regulate Ndel1/dynein activity and/or the structural conformation of vimentin. Alternatively, without affecting the activity of the complex, αCOP might simply use the Ndel1-dynein-vimentin complex as a docking site or as a carrier for its transport at the appropriate cellular compartment. On site, αCOP can then assemble with other members of the COPI complex to mediate its functions. Of particular note, consistent with our screen results, a recent report describes the recruitment of dynein to COPI vesicles via the GTP-binding protein Cdc42 (48). It remains unclear how Ndel1 cross-talks and/or co-operates with Cdc42 to regulate trafficking and IF dynamics. Further investigations are needed to verify all of these hypotheses.

*Ndel1 as Potential Target for Neurological Diseases Featuring Transport and Trafficking Defects*—Alterations in cytoskeletal proteins account for a wide diversity of neurodegenerative and neurodevelopmental disorders (7, 11, 12, 22, 49–51). In addition, there is a growing body of literature implicating molecules of transport and trafficking such as molecular motors, GTPase and vesicle-associated protein, as key targets during neuronal loss (52–56). Interestingly, many of these human mutated proteins such as NF-L, Lis1, dynactin, are direct Ndel1-binding partners (3, 5, 20–22, 51, 55–57), suggesting the existence of potential alterations (genetic or biochemical) in Ndel1 in neurological disorders. We previously reported a down-regulation of Ndel1 in a mouse model of amyotrophic lateral sclerosis and have extended this finding to other models of neurodegeneration.[4] Our findings that depletion of Ndel1 reduced vimentin motility and caused its accumulations further indicate that

[4] M. D. Nguyen and V. W. Yong, unpublished observations.

Ndel1 activity and/or function might be compromised in various transport/trafficking disorders (Fig. 4). The regulatory role of Ndel1 on vimentin dynamics may also be extrapolated to other IFs and in particularly to NF-H, which has been reported to associate with Ndel1-dynein-dynactin in central nervous system neurons (5, 17–19, 58). This idea is compatible with a report indicating that overexpression of the dynactin subunit p50 disrupts dynein activity and causes severe motor neuron degeneration associated with NF accumulations, reminiscent of amyotrophic lateral sclerosis. Of note, the down-regulation of Ndel1 in these models of neurodegeneration may be related to the role of Ndel1 in neurite outgrowth and may explain the inability of these axons to regenerate, despite up-regulation of vimentin (59, 60). This hypothesis would place Ndel1 at the interface of neurodevelopment, neurodegeneration, and neuroregeneration.

## CONCLUSION

In summary, our study unravels a novel regulatory mechanism of IF vimentin dynamics implicating Ndel1 during neurite outgrowth. Neurite outgrowth is often seen as a read-out for axon outgrowth during development and regeneration following injury. The absence of phenotype in vimentin null mice (61) strikingly contrasts with the embryonic lethality of Ndel1 null mice. Vimentin null mice exhibit impaired recovery of sensory response 6 days after lesions and reduced levels of GAP-43 (26). Thus, silencing Ndel1 selectively and locally in injured neurons may have more profound effects.

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# Ndel1 Controls the Dynein-mediated Transport of Vimentin during Neurite Outgrowth

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