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Identification of a Novel Dynein Binding Domain in Nudel Essential for Spindle Pole Organization in *Xenopus* Egg Extract*[S]

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The nuclear distribution protein E (NudE) and nuclear distribution protein E-like (Nudel or Ndel1) interact with both lissencephaly 1 (Lis1) and dynein. These interactions are thought to be essential for dynein function. Previous studies have shown that the highly conserved N terminus of NudE/Nudel directly binds to Lis1, and such binding is critical for dynein activity. By contrast, although the C terminus of NudE/Nudel was reported to bind to dynein, the functional significance of this binding has remained unclear. Using the sperm-mediated spindle assembly assay in *Xenopus* egg extracts and extensive mutagenesis studies, we have identified a highly conserved dynein binding domain within the first 80 amino acids of Nudel. We further demonstrate that the dynein intermediate chain in the dynein complex is directly involved in this interaction. Importantly, we show that both the dynein and Lis1 binding domains of Nudel are required for spindle pole organization. Finally, we report that spindle defects caused by immuno-depletion of Nudel could be rescued by a 1-fold increase of Lis1 concentration in *Xenopus* egg extracts. This suggests that an important function of the N terminus of Nudel is to facilitate the interaction between Lis1 and dynein during spindle assembly. Together, our findings open up new avenues to further decipher the mechanism of dynein regulation by Nudel and Lis1.

The cytoplasmic dynein is a minus-end-directed microtubule-based motor that regulates many cellular functions in interphase and mitosis, including membrane trafficking, nuclear migration, mitotic spindle assembly, and chromosome segregation. Dynein is a complex consisting of the catalytic dynein heavy chain and several non-catalytic subunits including dynein intermediate chain (DIC),[2] dynein light intermediate chain (DLIC), and dynein light chains (DLC). This complex is regulated by an array of proteins including dynactin, Bicaudal D, NudE/Nudel, and Lis1. These dynein regulators are critical to adapt dynein to membrane cargos or to other cellular components for transport along microtubules (1).

Among the dynein regulators, Lis1 is required for most aspects of dynein function. Lis1 binds to both the catalytic domain of the dynein heavy chain and several non-catalytic dynein subunits (2–4). The ability of Lis1 to regulate dynein is further controlled by its binding partner NudE/Nudel, which consists of a highly conserved N-terminal coiled-coil domain and an unstructured C terminus (5–7). The N-terminal coiled-coil domain mediates NudE or Nudel homo-dimerization and Lis1 binding (8), whereas the unstructured and evolutionarily less conserved C terminus interacts with a number of proteins including dynein heavy chain (3, 7, 9), lamin-B (10), neurofilament (11), Cdc42GAP (12), and focal adhesion kinase (13).

Because the C terminus of NudE/Nudel mediates interactions with multiple proteins, this region could be critical to link dynein to various cellular functions. For example, in mitosis the interaction between *Xenopus* Nudel and lamin-B helps to recruits lamin-B to microtubules to facilitate assembly of the lamin-B spindle matrix (14) in a dynein-dependent manner during spindle assembly (10). However, depletion of NudE and Nudel does not completely abolish lamin-B recruitment to microtubules (10), suggesting that additional dynein regulators, such as Bicaudal D (15), which can bind to lamin-B (16), could function redundantly with Nudel to recruit lamin-B to dynein. In addition, overexpressing the N-terminal coiled-coil region of the *Aspergillus* NUDE can rescue the NUDE null mutation (17). Moreover, the NudE/Nudel homolog in *Saccharomyces cerevisiae* does not contain the C-terminal region (18). Taken together, the above findings are consistent with the idea that the C terminus of NudE/Nudel functions redundantly with other dynein regulators.

The C-terminal dynein binding site on Nudel has also been proposed to be essential for dynein function (7, 19, 20). By interacting with Lis1 and dynein via its N and C termini, respectively, Nudel is thought to function as a bridge to bring dynein and Lis1 together (7, 19, 20). However, the nonessential function of the C terminus of NudE/Nudel homologous in fungi suggests that this C-terminal dynein binding site may not be essential for dynein regulation (17).

The spindle assembly assay in *Xenopus* cytostatic factor-arrested egg extracts offers an opportunity to study dynein regulation in mitosis without the complication of indirect

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[2] The abbreviations used are: DIC, dynein intermediate chain; DLIC, dynein light intermediate chain; DLC, dynein light chain.

effects caused by dynein mis-regulation in the previous interphase. Dynein is critical for spindle pole organization in both tissue culture cells (21) and *Xenopus* egg extracts (22). We have shown previously that depletion of NudE and Nudel in *Xenopus* egg extracts also causes defects in spindle pole focusing, which can be fully rescued using purified Nudel (10). This suggests that Nudel and most likely Lis1 regulate the function of dynein in spindle pole organization in mitosis. Using spindle pole focusing as an assay, we have identified an essential dynein binding domain within the N terminus of Nudel, right next to the previously identified Lis1 binding domain. We further demonstrate that both the dynein binding domain and the Lis1 binding domain of Nudel are required to regulate dynein function.

EXPERIMENTAL PROCEDURES

Cloning, Protein Expression, and Purification—The cDNAs encoding *Xenopus* Nudel (clone ID 6955255), dynein intermediate chain (clone ID 6861285), dynein light intermediate chain (clone ID 8074560), and dynein light chain 8 (clone ID 4057971) were purchased from Open Biosystems (Huntsville, AL). The PCR fragments corresponding to full-length or truncated open reading frames of the above proteins were subcloned into either pGEX6P2 or pET30a vectors at BamHI and NotI sites. Point mutations of Nudel were made using PCR site-directed mutagenesis method, and all point mutations were confirmed by DNA sequencing. One-liter cultures with A_{600} of 0.5–0.7 were induced with 24 μg/ml isopropyl 1-thio-β-D-galactopyranoside (PTG) for either 4 h at 37 °C or overnight at 22 °C. Bacteria were harvested by centrifugation and resuspended in 100 ml of TNGT buffer (20 mM Tris-HCl, pH 8.3, 0.8 M NaCl, 10% glycerol, and 0.2% Tween 20, 1 mM phenylmethylsulfonyl fluoride, and 100 μg/ml of lysozyme). The re-suspended bacterial cells were frozen at −80 °C overnight. Affinity purification using either glutathione-agarose beads (Sigma) or nickel-nitrilotriacetic acid resin (Qiagen) was carried out according to protocols provided by the manufacturers. The purified proteins were exchanged into XB buffer (10 mM Hepes, pH 7.7, 50 mM sucrose, 100 mM KCl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$, and 5 mM EGTA) using PD-10 column (GE Healthcare) and concentrated to desired concentrations with Amicon Ultra 30K device (Millipore). The protein concentration was determined by comparing the purified proteins with BSA standard on the same SDS-PAGE, then converting into molar concentrations with the calculated molecular weight. The N terminus of Nudel is highly soluble and can be stored as 100–250 μM stocks at −80 °C. The His-tagged full-length Nudel was further purified by passing through Mono Q column and stored in XB buffer at 3.4 μM. His-Lis1 was expressed in Sf9 cells by infecting with the recombinant baculovirus stock provided by Dr. Andrea Musacchio (European Institute of Oncology, Milan, Italy) and purified using nickel-nitrilotriacetic acid resin and stored as 60 μM stocks at −80 °C. All purified Nudel and Lis1 proteins retain their biological activities (as assayed by spindle pole organization) for more than 6 months at −80 °C.

Protein Pulldown Assay—20 μg of various GST fusion proteins or control GST protein was added to 100 μl of egg extracts. After incubation at 4 °C for 1 h, 20 μl of glutathione-agarose beads (bed volume) was added to the egg extracts and incubated for additional 30 min at 4 °C. The beads were washed 5 times with XB buffer for DIC pulldown. The beads was washed twice with XB buffer supplemented with 0.4 M NaCl and 0.5% Triton X-100 and twice with XB buffer for Lis1 pulldown. The beads were boiled in 40 μl of SDS sample buffer, and 5 μl of samples were loaded onto 10% SDS-PAGE. DIC was probed with monoclonal antibody 74.1 (Abcam, catalog no. ab23905, 1:1000 dilution), and Lis1 was probed with monoclonal antibody Lis1–338 (Sigma, catalog no. L7391, 1:2000 dilution).

Immuno-depletion and Sperm Spindle Assembly Assay—The cytostatic factor-arrested *Xenopus* egg extracts and sperms were prepared as previously described (24). Spindle assembly was carried out in a 25-μl reaction in which 22 μl of *Xenopus* egg extract was mixed with 1 μl of 25X sperm mix and 2 μl of purified proteins. The 25X sperm mix contains sperm at ~18,000 sperm/μl, 25X energy mix, and 1.5–2.0 mg/ml rhodamine-labeled tubulin. The reaction was carried out at room temperature for 100–120 min. All quantifications shown were done using the same time point of reaction. Nudel antibody was generated as previously described (10). Lis1 antibody was raised against purified GST-LisH domain (amino acids 1–99) of *Xenopus* Lis1 and was affinity-purified using His-LisH protein. Immuno-depletion was carried out by incubating 100 μl of egg extracts with 100 μg of Nudel antibody or Lis1 antibody coupled to 20 μl of Affi-prep protein A beads (bed volume) (Bio-Rad catalog no. 156-0005) at 4 °C for 1 h on a rotating shaker. To ensure sufficient contact of the egg extract with antibody beads, a few air bubbles were intentionally introduced into the extract during incubation. The beads were removed from the extract by centrifuging at 800 × *g* for 2 min at 4 °C. Two rounds of immuno-depletion are needed to remove the majority of Lis1, whereas one round of immuno-depletion is sufficient to remove the majority of Nudel.

Quantification of Spindle Morphology—Spindle pole focusing was quantified as dynein is required for the formation of focused bipolar spindles in *Xenopus* egg extracts. We have grouped assembled microtubule structures into four morphological categories, *i.e.* bipolar spindle, multi-polar spindle, partially focused spindle, and fence-like structure. Bipolar spindle is defined as structures with two focused poles and tightly aligned chromosomes near the equator of the spindle. Multipolar spindle is defined as structures with a narrowly aligned chromosome bar located near the equator, but microtubules are focused into more than two poles. Partially focused spindle includes structures with either one pole in focusing or neither pole in focusing. Loosely packed microtubules without pole focusing, but bent or tend to focus, are also included in this category. Fence is defined as microtubules aligning perpendicularly to the chromosome bar. These microtubules are usually straight and parallel with each other, and the chromosome bar is located in the middle of the microtubules. Each set of experiments was repeated with at least three different extracts prepared on separate days. 100–150 spindles were quantified in each sample.

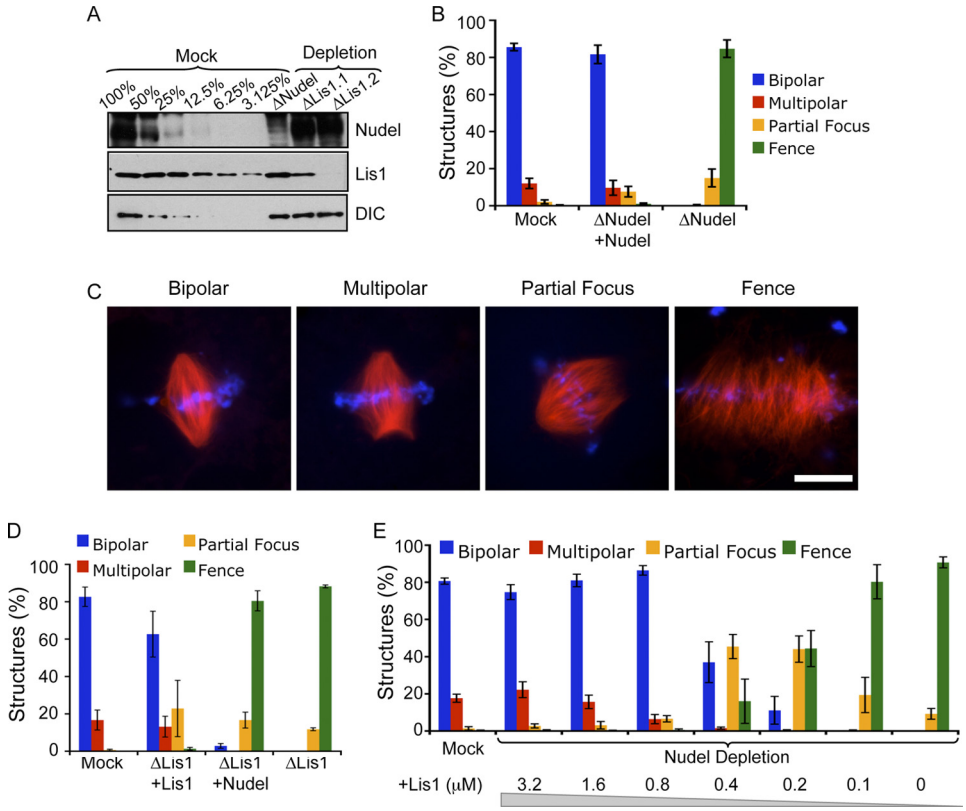

FIGURE 1. **Nudel functions to stimulate spindle pole focusing through Lis1.** *A*, immuno-depletion of Nudel from egg extracts is shown. One round of immuno-depletion is sufficient to remove most of Nudel/NudE in *Xenopus* egg extracts, whereas two rounds of immuno-depletions (Δ*Lis1.1* and Δ*Lis1.2*) routinely remove ~90% of Lis1 in egg extracts. Neither Nudel/NudE nor Lis1 depletion reduced the level of dynein as judged by Western blotting probing for the DIC. Control IgG-depleted egg extracts (*Mock*) were loaded at the indicated amount to assess the degree of immuno-depletion. *B*, spindle pole defects caused by Nudel/NudE depletion is fully rescued by adding purified Nudel protein. Immuno-depletion of Nudel resulted in the formation of mostly fence-like microtubules aligned perpendicularly across the sperm chromatin and a small fraction of spindles with multiple or partially focused poles (see the images in *C*), which can be fully rescued by addition of 50 nM concentrations of purified His-Nudel full-length protein. *C*, shown are representative images of spindles that are defined as bipolar, multipolar, partially focused, or parallel array of microtubules appearing as fence-like structure (*Fence*) perpendicular to the condensed chromatin. *D*, depletion of Lis1 caused a similar spindle pole defects as Nudel/NudE depletion. These defects could be rescued by adding 1 μM purified His-Lis1 but not by 1 μM His-Nudel. *E*, purified His-Lis1 fully rescues Nudel/NudE depletion. The addition of increasing concentrations of purified His-Lis1 shows that equal or greater than 0.8 μM His-Lis1 (the endogenous Lis1 is estimated at ~1 μM, see supplemental Fig. S1) is sufficient to fully rescue the spindle pole defects caused by Nudel/NudE depletion. *Error bar*, S.E. *Scale bars*, 20 μm.

S.E. was calculated as S.D. divided by the square root of the number of experiments (*n*).

RESULTS

Nudel Regulates Spindle Pole Organization through Lis1—We reasoned that the ability to study spindle assembly in *Xenopus* egg extracts using immuno-depletion and add-back could allow us to isolate one aspect of Nudel function in spindle pole organization. This may help us to gain a better understanding of how Nudel interacts with dynein and regulates dynein activity in the presence of a full complement of dynein regulators. We have shown previously that Nudel antibody immunodepletes both NudE and Nudel in *Xenopus* egg extracts (10). To further characterize Nudel function, we immuno-depleted NudE/Nudel from *Xenopus* egg extracts (Fig. 1*A*). Spindle assembly was induced using *Xenopus* sperm chromatin. As expected, whereas in mock-depleted egg extracts most spindles assembled are focused bipolar structures, depleting NudE/Nudel resulted in spindles having mostly unfocused poles with microtubules aligning perpendicularly to the sperm chromatin appearing as "picket fences" (*Fence* in Fig. 1, *B* and *C*). The addition of purified His-Nudel at the concentration equivalent to the endogenous Nudel/NudE (0.05–0.1 μM) (10) rescued the defects in spindle pole focusing (Fig. 1, *B* and *C*, and supplemental Fig. S1*A*).

To study whether Lis1 also functions to regulate spindle pole organization, we immuno-depleted Lis1 and found that Lis1 depletion pheno-copied NudE/Nudel depletion (Fig. 1, *A*, *C*, and *D*). Whereas the addition of purified His-Lis1 to the endogenous Lis1 concentration (~1 μM, see supplemental Fig. S1) rescued the spindle pole defects, the addition of 1 μM Nudel failed to do so (Fig. 1*D*). This shows that similar to Nudel, Lis1 is required for spindle pole organization. Moreover, the purified His-Lis1 can replace the function of endogenous Lis1.

To test whether Lis1 can replace NudE/Nudel function, we immuno-depleted NudE/Nudel and added an increasing concentration of Lis1 to egg extracts. We found that the addition of His-Lis1 to 0.8 μM (the endogenous Lis1 concentration is ~1 μM; see supplemental Fig. S1) is sufficient to rescue spindle pole defects (Fig. 1*E*). This shows that increasing Lis1 concentration by ~1-fold is sufficient to fully compensate for the defects caused by immuno-depletion of NudE/Nudel in spindle pole organization.

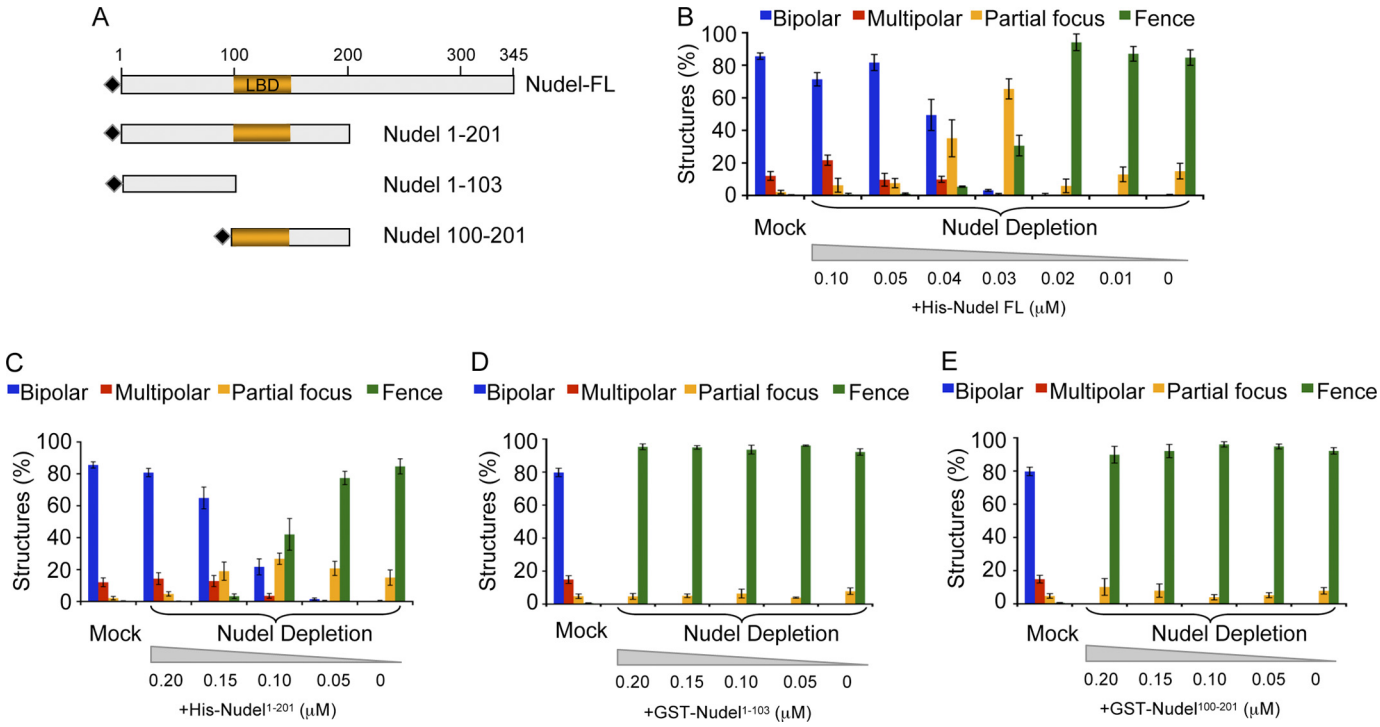

FIGURE 2. **The N-terminal coiled-coil domain of Nudel is sufficient for spindle pole organization when provided at 2–4-fold molar excess of the endogenous Nudel/NudE.** *A*, shown are schematic diagrams of Nudel full-length (*FL*) and fragments. *Xenopus* Nudel is 345 amino acids long with the Lis1 binding domain (*LBD*) localized between amino acids 100 and 153. All depicted Nudel proteins are tagged at their N termini with either GST or His (*black diamonds*). *B*, full-length His-Nudel rescues the spindle pole defects caused by Nudel/NudE depletion when added at equal or greater than 0.05 μM, which is similar to the estimated endogenous Nudel concentration (0.05–0.1 μM (10)). *C*, the N-terminal fragment containing the first 201 amino acids of Nudel can fully rescue the spindle pole defects caused by Nudel/NudE depletion when added at 0.2 μM, which is 2–4-fold higher than the endogenous Nudel/NudE concentration. Both His and GST-tagged Nudel$^{1-201}$ exhibit equivalent activities in this assay. Shown are quantifications for His-Nudel$^{1-201}$. *D* and *E*, the complete coiled-coil domain is required for spindle pole focusing. Neither the first 100 amino acids of Nudel that do not contain the Lis1 binding domain nor the second 100 amino acids of Nudel that contain the Lis1 binding domain can rescue spindle pole defects caused by Nudel/NudE depletion. *Error bars*, S.E.

The N Terminus of Nudel Has Spindle Pole-organizing Activity—Multiple functions have been assigned to the C terminus of NudE/Nudel in vertebrates, suggesting that this part of NudE/Nudel is important for its function. However, the C terminus of NudE/Nudel homologs in fungi are either nonessential (23) or largely missing from the protein (18). In *Aspergillus*, overexpressing the N-terminal coiled-coil region of NUDE is sufficient to rescue NUDE null phenotypes (23). By contrast, strongly overexpressing the N-terminal coiled-coil domain of Nudel causes dominant negative effects on dynein function in both mammalian tissue culture cells (6, 7) and *Xenopus* egg extracts (10). These observations prompted us to further examine the function of the N-terminal coiled-coil domain of Nudel.

We have shown that the addition of Nudel N-terminal 1–201 amino acids as a His-tagged protein (His-Nudel$^{1-201}$) (Fig. 2*A*) at ~50-fold molar excess of the endogenous NudE/Nudel disrupts formation of spindle-like structures stimulated by Aurora-A beads in egg extracts (10). To test whether the addition of a less amount of His-Nudel$^{1-201}$ could rescue NudE/Nudel depletion, as what was observed in *Aspergillus*, we immuno-depleted NudE/Nudel from egg extracts and added an increasing amount of His-Nudel$^{1-201}$ up to a 2–4-fold molar excess of the endogenous NudE/Nudel (10). Purified full-length Nudel (His-Nudel FL) was used as controls. As expected, the addition of 0.05–0.1 μM of the full-length Nudel rescued spindle pole focusing (Fig. 2*B*). Interestingly, we found that the addition of His-Nudel$^{1-201}$ at a 2–4-fold molar excess of endogenous NudE/Nudel also rescued spindle pole focusing defects caused by NudE/Nudel depletion (Fig. 2*C*). The same concentration of GST-tagged-Nudel$^{1-201}$ could also rescue spindle pole focusing (data not shown). This demonstrates that the N-terminal coiled-coil region of Nudel, when present at more than a 2-fold molar excess can compensate for the spindle pole defects caused by the reduction of NudE/Nudel.

The Lis1 Binding Activity at the N Terminus of Nudel Is Insufficient for Spindle Pole Focusing—Previous studies have suggested that Lis1 binding is important for NudE/Nudel to regulate dynein. The Lis1 binding domain has been mapped to within the N-terminal 100–153 amino acids of NudE/Nudel. To further define the spindle pole focusing activity present in the N-terminal coiled-coil region of Nudel, we created two GST fusion fragments of Nudel that either contain or lack the Lis1 binding domain (Fig. 2*A*). We found that none of these fragments was able to rescue the spindle pole focusing defects caused by NudE/Nudel depletion (Fig. 2, *D* and *E*). This suggests that some activities in addition to Lis1 binding are required for the N terminus of Nudel to regulate spindle pole organization.

Identification of a Novel DIC Binding Domain within the N Terminus of Nudel—Because the interaction between NudE/Nudel and dynein has been suggested to be important for dynein regulation, we suspected that there could be a dynein

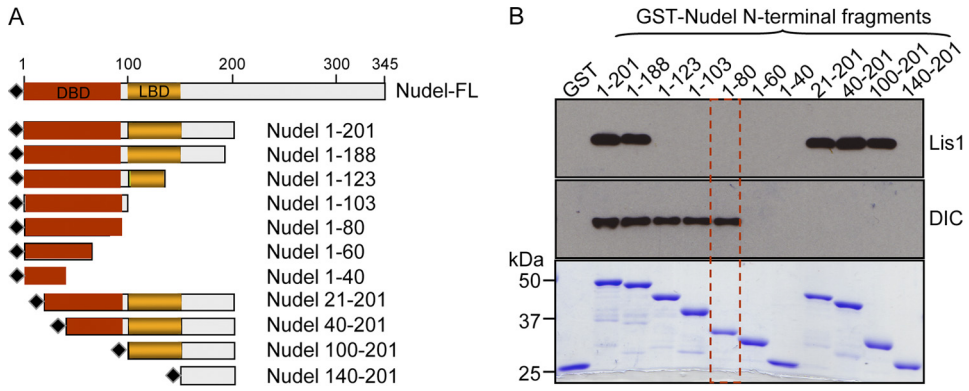

FIGURE 3. **Identification of a dynein binding domain in the N terminus of Nudel.** *A*, schematic diagram of *Xenopus* Nudel with deletion series from either N- or C terminus is shown. *DBD*, dynein binding domain. *LBD*, Lis1 binding domain. All proteins were tagged by GST (*black diamonds*). *B*, the purified GST-tagged Nudel fragments and their ability to interact with either dynein or Lis1 in *Xenopus* egg extracts is shown. Purified GST was used as controls. The minimal dynein binding region was mapped to the first 80 amino acids (1–80 as indicated by a *red dashed square*). As expected the minimal Lis1 binding domain is mapped to amino acids 100–153 of Nudel.

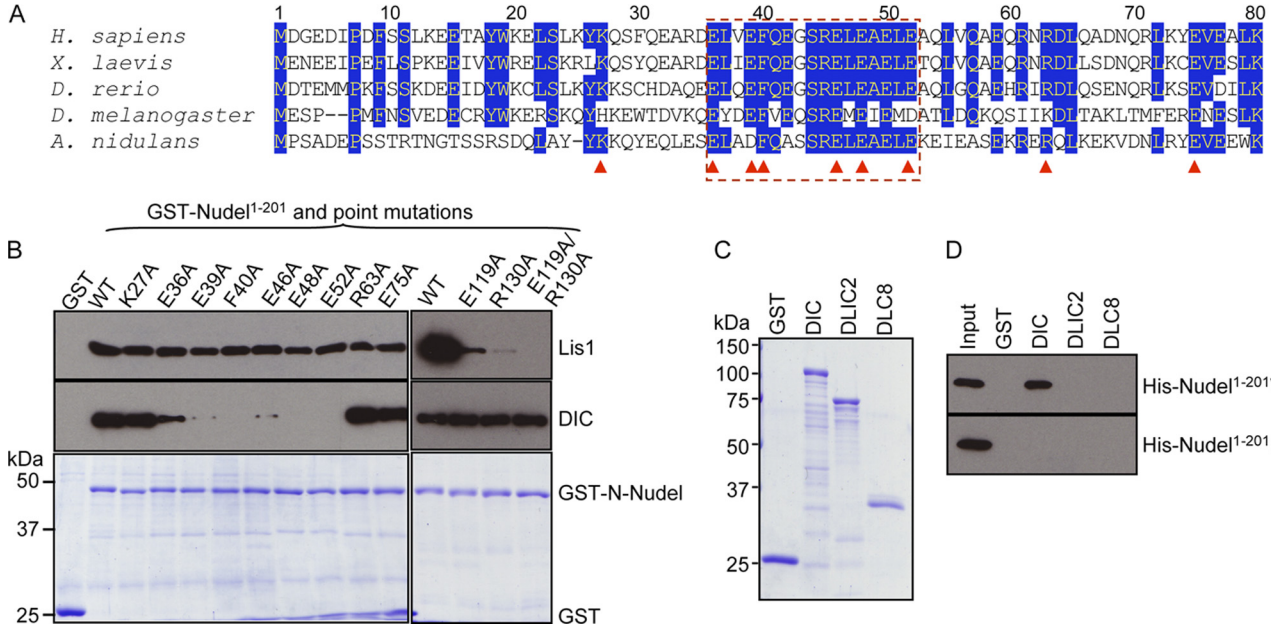

FIGURE 4. **Nudel directly interacts with the dynein intermediate chain.** *A*, shown is sequence alignment of the N-terminal 80 amino acids of Nudel from different organisms (*numbers* are for *Xenopus* Nudel). Amino acids mutated to alanine are indicated by *red arrowheads*. The highly conserved region is *outlined by a red dashed square*. *B*, highly conserved amino acids in Nudel mediate dynein binding. The highly conserved amino acids as indicated in *A* are mutated to alanines in GST-Nudel$^{1-201}$. Point mutations of conserved amino acids greatly reduced dynein binding without affecting Lis1 binding. Point mutations in the Lis1 binding domain diminishes Lis1 binding without affecting dynein binding. *C*, shown is SDS-PAGE of purified GST, DIC, DLIC2, and DLC8 stained by Coomassie Blue. *D*, Nudel directly binds to DIC. Purified His-Nudel$^{1-201}$, but not purified His-Nudel$^{1-201}$E48A, binds purified DIC.

binding domain within the N-terminal coiled-coil region of Nudel. To examine this, we first carried out deletion analyses from either the N or C terminus of GST-Nudel$^{1-201}$ (Fig. 3*A*). A total of 11 GST-tagged fragments was expressed and purified from bacteria (see the Coomassie Blue-stained gel at the *bottom* of Fig. 3*B*). Each of these fragments was individually added to egg extracts to test their ability to bind to dynein and Lis1 using protein pulldown assays. Using antibodies to Lis1 and DIC, we found a dynein binding domain localized within the first 80 amino acids of Nudel (Fig. 3*B*). Whereas this dynein binding domain does not mediate Lis1 binding, fragments containing the previous mapped Lis1 binding domain (amino acids 100–153) all bind to Lis1 but not to dynein as expected (Fig. 3*B*).

To further characterize this newly identified dynein binding domain, we aligned this region using Nudel sequences from evolutionarily divergent organisms (Fig. 4*A*). We individually replaced nine highly conserved amino acids in this region with alanine to create GST-Nudel$^{1-201}$ fusion proteins carrying point mutations. We also created point mutations in the Lis1 binding domain that are known to affect Lis1 binding in GST-Nudel$^{1-201}$ (Fig. 4*B*). Using protein pulldown assays, we found that the conserved amino acids Glu-36, Glu-39, Phe-40, Glu-46, Glu-48, and Glu-52 within a highly conserved region of Nudel are required for dynein binding, whereas amino acids Lys-27, Arg-63, and Glu-75 outside of this region do not play a significant role (Fig. 4*B*). The single point mutations E119A and R130A exhibit a great reduction in Lis1

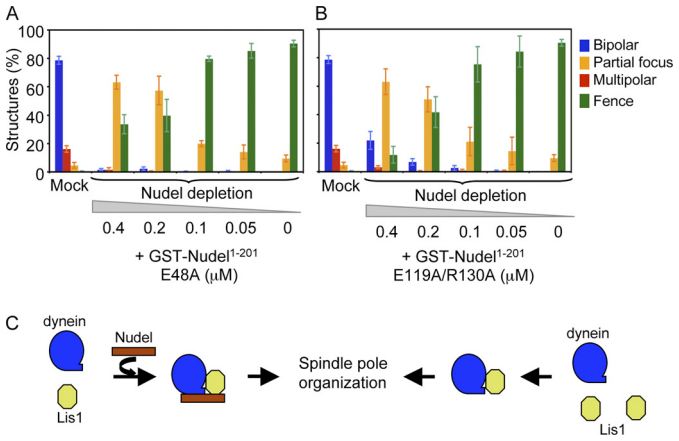

FIGURE 5. **The N-terminal dynein and Lis1 binding domains are both essential for spindle pole organization.** *A*, the GST-Nudel$^{1-201}$E48A that does not bind to DIC fails to rescue spindle pole defects caused by Nudel/NudE depletion. *B*, the GST-Nudel$^{1-201}$E119A/R130A that fails to bind to Lis1 also does not rescue spindle pole defects. *C*, shown is a model depicting how Nudel functions to regulate dynein through Lis1. Nudel functions as a bridge to facilitate Lis1 and dynein interaction. Without Nudel, an increase of Lis1 concentration is sufficient to promote Lis1 and dynein interaction for spindle pole organization. *Error bars*, S.E.

binding, whereas GST-Nudel$^{1-201}$ containing the double E119A/R130A mutations has no detectable Lis1 binding under the same conditions (Fig. 4*B*).

To further determine whether one of the dynein non-catalytic subunits might directly bind to this region of Nudel, we expressed and purified the *Xenopus* DIC, DLIC2, and DLC8 from bacteria (Fig. 4*C*). We found that purified Nudel only interacted with the purified DIC but not DLIC2 or DLC8 (Fig. 4*D*). Moreover, the His-Nudel$^{1-201}$E48A mutant known to not pull down dynein in *Xenopus* egg extracts also failed to bind to DIC (Fig. 4*D*). Therefore, the N terminus of Nudel binds to dynein through DIC.

Both the Dynein and Lis1 Binding Domains in the N Terminus of Nudel Are required for Spindle Pole Organization—Using the spindle assembly assay, we found that the point mutation in Nudel that abolishes dynein binding also abolishes the ability of GST- Nudel$^{1-201}$ to rescue spindle pole defects due to NudE/Nudel depletion (Fig. 5*A*). This demonstrates that the dynein binding domain in the first 80 amino acids of Nudel is essential for spindle pole organization. As expected, point mutations in Lis1 binding domain abolished the ability of GST-Nudel$^{1-201}$ to fully rescue spindle pole organization (Fig. 5*B*). Therefore, both the dynein binding and the Lis1 binding domains of Nudel are required for spindle pole assembly. Together the above findings suggest that Nudel functions to facilitate the interaction between Lis1 and dynein (Fig. 5*C*).

DISCUSSION

The spindle assembly assay in *Xenopus* egg extracts offers an opportunity to study dynein function in the presence of a full complement of regulators. By assaying for the pole morphology of spindles induced by *Xenopus* sperm DNA, we demonstrate that the N-terminal 1–201 amino acids of Nudel, consisting of the newly identified dynein binding domain and the previously defined Lis1-binding domain, is sufficient to regulate dynein function in spindle pole focusing when present at 2–4-fold higher molar concentrations than the endogenous Nudel. The C terminus of Nudel has been found to bind to a number of proteins including dynein and lamin-B. In the absence of the C terminus of Nudel, some of these proteins, such as lamin-B, could be recruited to dynein by other dynein adaptors such as Bicaudal D (16) to facilitate spindle matrix assembly.

Because the only known dynein binding site in Nudel was mapped to the C terminus of Nudel, it has been hypothesized that this C-terminal dynein binding site works with the N-terminal Lis1 binding domain to facilitate Lis1 and dynein interaction. However, we have defined a new dynein binding domain within the first 80 amino acids of Nudel right next to the previously defined Lis1-binding domain. Previous studies suggested that NudE interacts with the intact dynein through DIC and DLC8 (7). Here we show that the N terminus of Nudel binds directly to DIC but not to DLC and DLIC. More importantly, we demonstrate that both the N-terminal dynein binding domain and the Lis1 binding domain are essential to mediate spindle pole organization. This shows that Nudel and NudE use their N terminus coiled-coil regions to facilitate the interaction between dynein and Lis1. Our findings strongly suggest that Nudel functions as a bridge to bring dynein and Lis1 into close proximity, which would facilitate Lis1 to bind to dynein to regulate dynein activity (Fig. 5*C*). We found that depletion of Nudel could be rescued by simply increasing Lis1 concentration in the egg extracts. An elevated Lis1 concentration would enhance Lis1 and dynein interaction, consequently circumventing Nudel deficiency (Fig. 5*C* and see more below). These findings provide important new insights that should allow proper modeling of how Nudel can hold Lis1 in the close proximity of dynein to facilitate dynein function (20).

We showed that spindle pole defects caused by a >90% reduction of Nudel/NudE levels can be rescued by an approximately 1-fold increase of Lis1 protein concentration. Therefore, in the context of spindle pole assembly, increasing Lis1 concentration is sufficient to allow Lis1 to bind to dynein in a correct configuration for function. However, Nudel has been shown to silence dynein by inhibiting Lis1-stimulated binding of dynein to microtubules *in vitro* (20). Our findings suggest that either the residual Nudel/NudE or additional dynein regulators in the egg extract might fulfill such a function if Lis1 concentration is increased. Considering that Lis1 overexpression in *Aspergillus* can rescue the NUDE null mutant, we favor the later possibility. Further *in vitro* studies using purified dynein, Nudel$^{1-201}$, and Lis1 will be needed to reveal how the N-terminal DIC binding domain of Nudel facilitates Lis1 and dynein interaction and dynein force production on microtubules assembled *in vitro*.

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