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Review

Nicholas J. Bradshaw*, William Hennah and Dinesh C. Soares*

NDE1 and NDEL1: twin neurodevelopmental proteins with similar ‘nature’ but different ‘nurture’

Abstract: Nuclear distribution element 1 (NDE1, also known as NudE) and NDE-like 1 (NDEL1, also known as Nudel) are paralogous proteins essential for mitosis and neurodevelopment that have been implicated in psychiatric and neurodevelopmental disorders. The two proteins possess high sequence similarity and have been shown to physically interact with one another. Numerous lines of experimental evidence *in vivo* and in cell culture have demonstrated that these proteins share common functions, although instances of differing functions between the two have recently emerged. We review the key aspects of NDE1 and NDEL1 in terms of recent advances in structure elucidation and cellular function, with an emphasis on their differing mechanisms of post-translational modification. Based on a review of the literature and bioinformatics assessment, we advance the concept that the twin proteins NDE1 and NDEL1, while sharing a similar ‘nature’ in terms of their structure and basic functions, appear to be different in their ‘nurture’, the manner in which they are regulated both in terms of expression and of post-translational modification within the cell. These differences are likely to be of significant importance in understanding the specific roles of NDE1 and NDEL1 in neurodevelopment and disease.

Keywords: NDE1/NudE; NDEL1/Nudel; neurodevelopment; phosphorylation; post-translational modification.

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Introduction

Nuclear distribution element 1 (NDE1, also known as NudE) and NDE-like 1 (NDEL1, also known as Nudel) are a pair of highly similar coiled-coil-containing proteins (Figure 1), believed to have evolved from a common ancestral gene, such as the *NudE* gene of *Aspergillus nidulans*, after which they were named (1–5). Both of these proteins are known to be critical for neurodevelopment and have been implicated in a range of mental health measures and neurodevelopmental conditions. Copy number variations (CNVs) at the 16p13.11 chromosomal locus, that contains the *NDE1* gene among others, have been associated with intellectual disability, autism, attention deficit hyperactivity disorder, schizophrenia and epilepsy (6–18). In contrast, the region of the 17p13.1 locus where *NDEL1* resides has not been directly implicated in brain disorders through CNV analysis. However, it should be noted that genomic structural variation at the 16p13.11 locus is more common than at 17p13.1, even among healthy individuals [database of genomic variants; ref. (19)]. Of the genes disrupted by CNVs at 16p13.11, disruption of normal *NDE1* function stands out as the most likely cause of the associated mental health problems due to the known functional roles of the NDE1 and NDEL1 proteins through interaction with Disrupted In Schizophrenia 1 (DISC1), a molecule strongly implicated in risk of mental illness (reviewed in refs. 20–23).

The *DISC1* gene was discovered because it is directly disrupted by a balanced translocation that segregates with major mental illness in a single large Scottish pedigree (24, 25). Initial efforts to characterize DISC1 function revolved around attempts to discover its protein interaction partners, on the assumption that such knowledge would suggest biological functions in which the novel and unique DISC1 protein might partake. This led to the formation of the DISC1 pathway hypothesis, which hypothesized that disruption of components of the DISC1 pathway could influence susceptibility to schizophrenia and related disorders (26, 27). NDE1 and NDEL1 were both

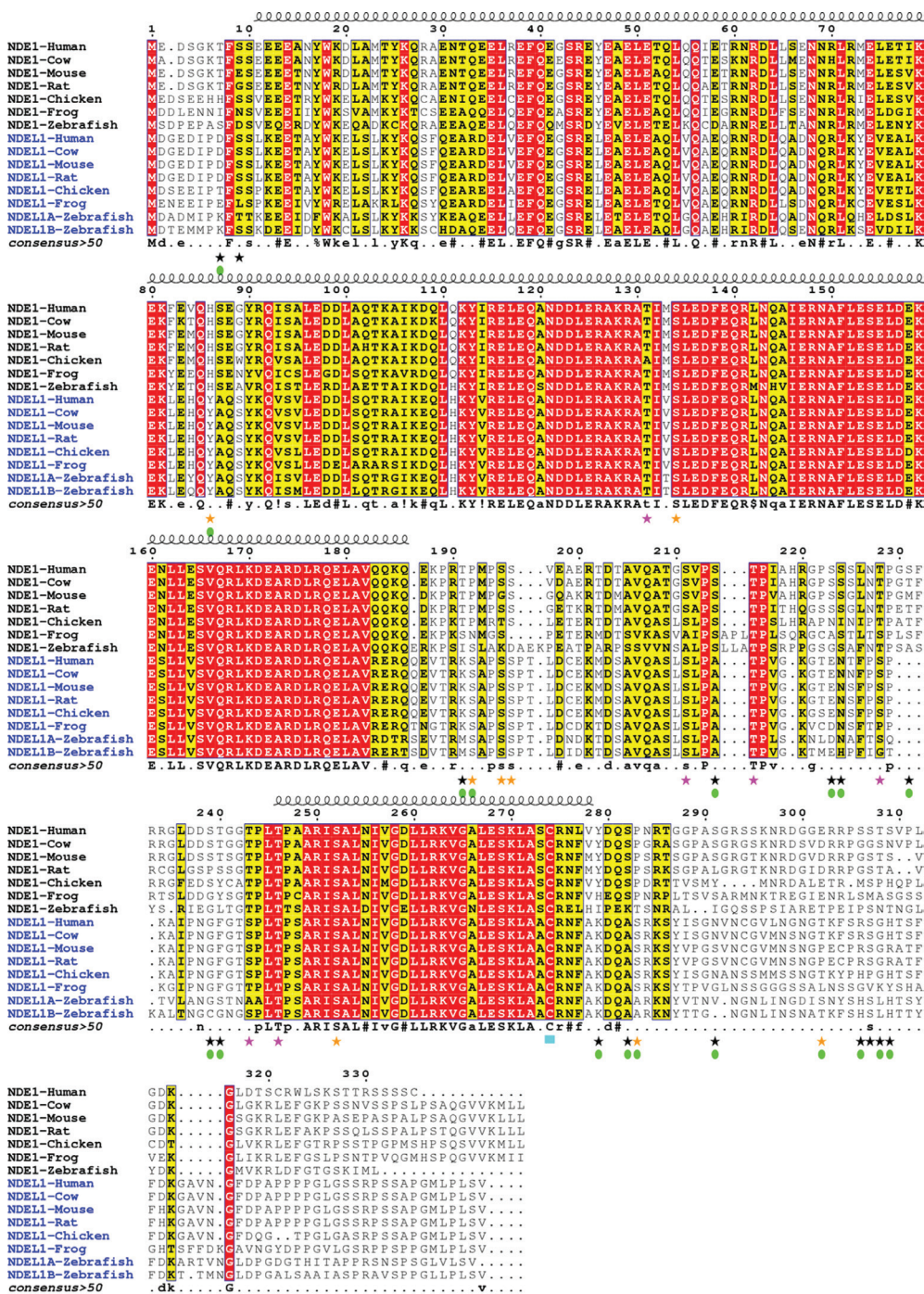


Figure 1 Multiple sequence alignment of NDE1 and NDEL1 orthologs. Orthologs of NDE1 and NDEL1 across vertebrate species were identified using the UCSC Genome Browser (<http://genome-euro.ucsc.edu>). These were aligned initially using Clustal Omega (108, 109) with further manual editing for optimum alignment. The sequence numbering at the top of each alignment block corresponds to the sequence of human NDE1. Conserved residues are colored with a red background; conservatively substituted residues are shown with a yellow background. The location of the N-terminal coiled-coil α -helical domain (residues ~10–185) and the predicted C-terminal α -helix (residues ~247–278) are shown above the alignment. Consensus amino acids are shown at the bottom of the each alignment block (uppercase is strictly conserved; lowercase is consensus level >0.5; ! is I or V; \$ is L or M; % is F or Y; = is N, D, Q or E). Despite 60% sequence identity there are at least 18 specific phosphorylation sites for each human protein – i.e., phosphorylation occurs only in one protein. Some of these phosphorylation sites have been derived from high-throughput proteomic screening using mass spectrometry (90), while others have been experimentally verified in the cell. See the main text and Table 2 for more information. Those sites known to be specific to either NDE1 or NDEL1 are highlighted with a green filled oval under the corresponding star; black star=phosphorylation site in NDE1; orange star=phosphorylation site in NDEL1; pink star=phosphorylation site in both NDE1 and NDEL1; cyan block=palmitoylation site in both proteins. The figure was generated with ESPrnt v2.2 (111).

found to directly interact with DISC1 (28–33) and have since become central to this hypothesis (20–23).

In genetic association studies of *NDE1* and *NDEL1* using single-nucleotide polymorphisms and haplotypes, both genes have provided evidence for involvement in mental illness phenotypes, with significant association ascertained independently in a Finnish family cohort for schizophrenia (26, 34), and in a Caucasian-American schizophrenia cohort where the association depended on the status of a common variant in DISC1 (C704) (29). A variant at the *NDEL1* locus has also been demonstrated to associate with schizophrenia in interaction with an independently significant variant from the *CIT* gene, encoding another DISC1 interacting protein (35). However, such significant associations have not been uniformly observed (35–39), nor have they yet been found to be significant in genome-wide association studies (40).

Deletions of the 16p13.11 locus are also associated with mild microcephaly (7). Again, *NDE1* stands out as the most likely gene to be responsible for the greater part of the effect, as extreme microcephaly with lissencephaly was reported in multiple patients with biallelic frameshift mutations leading to truncation of the NDE1 protein (41, 42). Additionally, a biallelic mutant predicted to abolish NDE1 expression has been reported in patients with microhydranencephaly (43). A recent study investigating association of the 16p13.11 region with microcephaly and a fetal brain disruption-like phenotype also found a similar phenotype to occur in two patients with deletion of one copy of 16p13.11, combined with a frameshift mutation in the remaining *NDE1* gene (44).

Therefore, genetic association evidence exists implicating both *NDE1* and *NDEL1* in psychiatric illness, with additional evidence from CNV and gene-disrupting mutations implicating *NDE1* specifically in a wider array of neurodevelopmental conditions. In this review, we assess both proteins, with a focus on what properties they share in common, but also the manner in which their regulation and functions differ – differences that may help explain their specific roles in neurodevelopment and disease and why two highly similar proteins have been retained in vertebrate evolution.

NDE1 and NDEL1 play overlapping roles in the cell and developing brain

NDE1 and NDEL1 were initially identified as part of a highly evolutionarily conserved pathway consisting of

the motor protein dynein and its various ancillary and regulatory proteins (1–5). These included the developmentally critical Lissencephaly 1 protein [LIS1, encoded by the *PFAH1B1* gene; ref. (45)]. NDE1 is known to recruit LIS1 to dynein, where they together induce a force-state, facilitating the movement of dynein along microtubules. In contrast, on its own NDE1 inhibits dynein motility by causing it to dissociate from the microtubules (46). NDEL1 appears to act in the same way, with its LIS1-binding N-terminus stimulating the movement of dynein, while its C-terminus, expressed alone, causes dynein-microtubule dissociation (47). Interestingly, while both proteins can interact with the intermediate chain of the dynein complex (2, 48), NDEL1, but not NDE1, can also bind the motor domain of dynein, in its heavy-chain (1, 2, 46). Additionally, NDE1 is known to compete with dynactin (dynein-activator) for binding to the dynein intermediate chain, and possesses a distinct site for interaction with dynein light chain 8 (LC8) (49). NDEL1 is not known to share this latter feature, and the LC8 binding region of NDE1 (residues 200–203) is only partially conserved in NDEL1 (Figure 1).

NDEL1 is known to affect many aspects of dynein function, including transport along the microtubule network of vesicles (50, 51) and lysosomes (52), with effects on the correct structure of the Golgi apparatus, as well as on the transport of intermediate filament proteins (53), short microtubules and viral glycoproteins (54). Of these known roles of NDEL1, NDE1 has also been shown to affect the size of the Golgi apparatus, with knockdown of NDE1 in HeLaM cells having a smaller effect than NDEL1, but with the double-knockdown having a greater effect still, implying that they can partially compensate for each other's loss (55). Peptides derived from each of NDE1 and NDEL1 also affect actin transport in a squid axonal model (56).

NDE1 and NDEL1 have also been demonstrated to be of significant importance in mitosis, with *Nde1* knockout mice displaying frequent instances of misaligned spindles in their cortical progenitor cells (57). Use of an antibody against both NDE1 and NDEL1 to disrupt their function induces similar defects, and disrupts the dynein-related removal of proteins from the kinetochore essential for mitotic progression at metaphase (48). Interestingly, this effect may be mediated by NDE1 alone, as suppression of *NDE1*, but not *NDEL1*, stopped dynein from localizing to the kinetochore and the cells from passing this mitotic checkpoint (58). This NDE1-specific role has, however, been contradicted by another study (59), while expression of *Xenopus* Ndel1 alone was sufficient to rescue spindle alignment defects caused by depletion of endogenous Nde1 and Ndel1 from egg extracts (60). NDEL1 has also been shown to stabilize dynein at the kinetochore (61) as

well as having additional mitotic roles in the breakdown of the nuclear envelope (62) and the assembly of the lamin B spindle matrix (63). NDE1 also has a dynein LC8-related effect on ciliogenesis during mitosis (64).

Nde1 knockout mice display thinning of the superficial layers of the cerebral cortex leading to a reduction in brain volume (57) reminiscent, but not as severe as, the microcephaly displayed in human patients with non-functional *NDE1* genes (41, 42). This appears to occur due to defects in neuronal migration and neurogenesis amongst neuronal progenitors, potentially as a result of incorrect orientation of the spindle during cell division (57). Additional effects of mouse *Nde1* on cortical neurodevelopment in conjunction with *Lis1* via radial glial cell function and spatial co-ordination of MAPK signaling pathways have also been described (65, 66).

In contrast *Ndel1* knockout mice are not viable, with blastocysts perishing within days of fertilization (51). Studies depleting the level of *Ndel1* in the developing mouse by various means, have also demonstrated defects in cortical neuronal migration (51, 67–69) leading to a thinning of the cortex (69) and implying an overlapping role with NDE1. This seemingly occurs due to uncoupling of the centrosome from the nucleus (68), an effect which is exaggerated when *Lis1* expression is also knocked-out (51, 69). Aberrant positioning of both embryonic and adult-born hippocampal neurons (51, 69, 70) and of neurite extension in dorsal root ganglia (71) have also been reported following NDEL1 depletion.

A number of additional functions for NDEL1 have also been reported including its facilitation of neurofilament polymerization (72), promotion of axonal regeneration (73), regulation of Cdc42 at the leading edge of migrating neurons (74), regulating the GTPase activity of the microtubule remodeling protein Dynamin 2 (75), facilitating the actin polymerization-promoting activity of the WAVE complex (76) and a peptidase activity (77). It is not currently known whether NDE1 shares any of these functions. For more detail on the cytoskeletal functions of NDEL1 and its role in signaling pathways, readers are referred to the comprehensive review by Chansard et al. (78).

It is therefore apparent that, in terms of the regulation of dynein, effects on mitotic progression and regulation of cortical neuronal development and migration, NDE1 and NDEL1 possess functions which largely overlap and that the two proteins are able, to some extent, to compensate for the loss of one another. Several lines of evidence suggest that the two proteins are not fully redundant, however, notably the potential differences in their dynein-related regulation of mitotic checkpoints (58),

overlapping but distinct regions of binding to the dynein protein complex (1, 2, 46, 49), the difference in viability of *Nde1* and *Ndel1* knockout mice (51, 57) and the dramatic microcephaly phenotypes seen in patients with loss of functional NDE1, but presumably not functional NDEL1 (41–44). There also remain a large number of functions of one of the paralogs, for which the importance of the other is undetermined. There are also notable differences in the expression of the two proteins over development, at the mRNA level (Figure 2). According to data from the Human Brain Transcriptome project [hbatlas.org; ref. (79)], while expression of *NDEL1* across the brain is relatively steady throughout the lifetime, *NDE1* is considerably higher during the first couple of months after fertilization, before dropping dramatically at birth. A smaller increase in *NDE1* expression is also notable in some brain regions in early childhood. Given the high degree of amino acid conservation between NDE1 and NDEL1 (Figure 1; Table 1), we will now discuss the potential basis of some of these similarities and differences: how do their structures compare? Do the NDE1 and NDEL1 proteins function differently, or are they proteins with a common function, but with distinct regulation?

NDE1 and NDEL1 have a similar nature: sequence, structure and oligomeric state

At the sequence level, NDE1 and NDEL1 share approximately 60% amino acid sequence identity (~80% sequence similarity) and are thought to have evolved from a common ancestral gene (1–5). This duplication and divergence into two genes appears to have occurred around the time of the emergence of vertebrate species (54), with NDEL1 orthologs showing greater inter-species conservation compared to NDE1 orthologs (Table 1). Multiple isoforms for both proteins have been confirmed, mainly consisting of a variety of alternative extreme C-terminal regions (33). Both evolutionarily conserved proteins have a highly conserved N-terminal half (residues ~1–185), while the C-terminal sequence is comparatively more variable (Figure 1). Sequence analysis revealed predominance of the classical ‘heptad’ repeat that could confer the coiled-coil motif to the N-terminal ~190 amino acids (80).

Early structure-based work focused exclusively on NDEL1. Sasaki et al. showed that full-length murine *Ndel1* interacted strongly with itself, and by means of yeast two-hybrid based assays using NDEL1 truncation

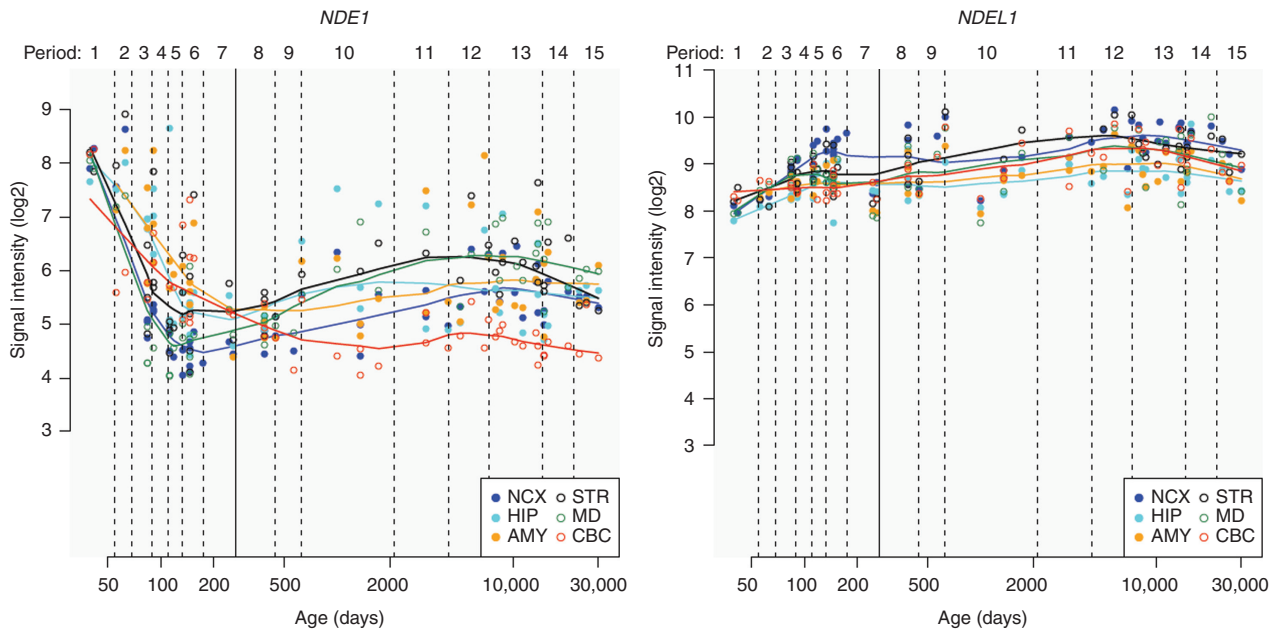


Figure 2 Differential expression of *NDE1* and *NDEL1* transcripts in the human brain across the lifetime. Change in *NDE1* mRNA expression with time is shown in the left panel and *NDEL1* in the right panel; developmental age shown on the x-axis in days; mRNA expression signal intensity (Log2) shown on the y-axis. While, expression of *NDEL1* across the brain remains relatively steady, *NDE1* shows a far more specialized pattern of expression. Abbreviations for brain regions used in figure: NCX, neocortex; STR, striatum; HIP, hippocampus; MD, mediodorsal nucleus of the thalamus; AMY, amygdala; CBC, cerebellar cortex. Data and figure accessed from the Human Brain Transcriptome project (<http://hbatlas.org/pages/hbtd>; used with permission) (79).

Table 1 Sequence comparison of *NDE1* and *NDEL1* shows greater conservation of *NDEL1* across vertebrate species.

Cow	88.66					Amino acid conservation in <i>NDE1</i> (% identity)
(<i>Bos taurus</i>)	99.13					Amino acid conservation in <i>NDEL1</i> (% identity)
	-10.47					Difference in conservation (% identity)
Mouse	85.89	87.50				
(<i>Mus musculus</i>)	95.94	96.52				
	-10.05	-9.02				
Rat	83.13	84.59	91.57			
(<i>Rattus norvegicus</i>)	95.94	96.52	100.00			
	-12.81	-11.93	-8.43			
Chicken	73.33	73.90	73.45	72.27		
(<i>Gallus gallus</i>)	92.13	92.71	90.67	90.67		
	-18.80	-18.81	-17.22	-18.40		
Frog	68.56	66.96	67.06	65.60	67.74	
(<i>Xenopus tropicalis</i>)	81.23	81.52	80.35	80.35	83.48	
	-12.67	-13.56	-13.29	-14.75	-15.74	
Zebrafish	58.54	57.93	57.98	58.28	58.95	56.50
(<i>Danio rerio</i>)	72.19/76.33	71.89/76.04	70.41/74.56	70.71/74.56	71.73/75.30	69.03/71.98
	-13.65/-17.79	-13.96/-18.11	-12.43/-16.58	-12.13/-16.28	-12.78/-16.35	-12.53/-15.48
Human		Cow	Mouse	Rat	Chicken	Frog
(<i>Homo sapiens</i>)		(<i>Bos taurus</i>)	(<i>Mus musculus</i>)	(<i>Rattus norvegicus</i>)	(<i>Gallus gallus</i>)	(<i>Xenopus tropicalis</i>)

The percentage amino acid sequence identity was compared using Clustal Omega (108, 109) after alignment shown in Figure 1. All-against-all pairwise inter-species percentage identity for *NDE1* is shown on the top row on a lighter gray background, and for *NDEL1* on the middle row on a white background. The difference between these values is shown on the bottom row in bold type on a darker gray background. As there are two *NDEL1* genes in zebrafish (110), values for both encoded proteins are displayed, *ndel1a* on the left and *ndel1b* on the right. It can be seen that in every instance, between all species examined, *NDEL1* is more highly conserved than *NDE1*.

constructs, showed that this potential ‘homodimerization’ resided within the putative coiled-coil domain (amino acids 56–166) (1). The emergence of high-resolution crystal structures and biophysical characterization of an N-terminal coiled-coil domain fragment for rat Ndel1 (identical in amino acid sequence to human NDEL1 for the region solved) (81) revealed a highly extended, continuous, tightly associating coiled-coil protein that facilitated both parallel dimerization and anti-parallel tetramerization (Figure 3); the latter composed of two parallel dimers associating in anti-parallel fashion. Initial chemical cross-linking analysis coupled with mass spectrometry (MS) supported the parallel dimer arrangement of NDEL1 in solution (82). High molecular weight-only species (~400 kDa) for His₆-tagged recombinant NDEL1 were also noted (83), while predominantly tetrameric species of GB1-tagged NDEL1 were described by Narayanan et al. using size-exclusion chromatography and analytical ultracentrifugation (84). Tarricone et al. (85) showed that a dimer of NDEL1 was important for interaction with LIS1, and Narayanan et al. (84) suggested that a stable tetramer was responsible for interaction with DISC1. Sequence-analysis and homology-based modeling suggested that NDE1 could indeed form both the dimeric and tetrameric species, and coiled-coil orientation (86), as observed in the equivalent NDEL1 crystal structures (81). Monomeric (~40 kDa) species of NDEL1 with a cysteine peptidase activity and composed of ~40% β -sheet by circular dichroism have also been reported (77), but are inconsistent with the remainder of the biophysical literature on NDEL1.

Our more recent biophysical characterization using size-exclusion chromatography, circular dichroism, negative-stain electron microscopy, and chemical cross-linking coupled with MS analysis and bioinformatics confirmed that both N-terminally His₆-tagged NDE1 and NDEL1 exhibit similar secondary structure, tertiary structure and oligomeric state (87). Both proteins form dimers, tetramers and high molecular weight species in solution thus unifying disparate observations in the literature. The application of chemical cross-linking/MS methodology allowed investigation of the C-terminal regions and thus provided insight into the conformation and overall architecture of the native proteins and was the first biophysical study that investigated the structure of NDE1. The C-terminal portion of the proteins was shown to ‘bend-back’, potentially using the proline-rich region in the C-terminal half of the protein that is predicted to be unstructured (88), and interact with the N-terminal coiled-coil domain (87). This result was consistent with functional observations of two disparate dynein binding sites on NDEL1 at its N- and C-termini (60, 89). Dynamic change in conformation for NDEL1 was also previously observed (90), in keeping with the flexible role of the C-terminus. We also showed via a novel application of stable-isotope labeling in cell culture (SILAC) chemical cross-linking and MS that NDE1 can directly and physically interact with NDEL1 through the formation of heterotetramers and higher oligomeric species (87). This is in agreement with the observation that the two proteins exist within the same protein complex in the cell, as determined by co-immunoprecipitation experiments (29, 33).

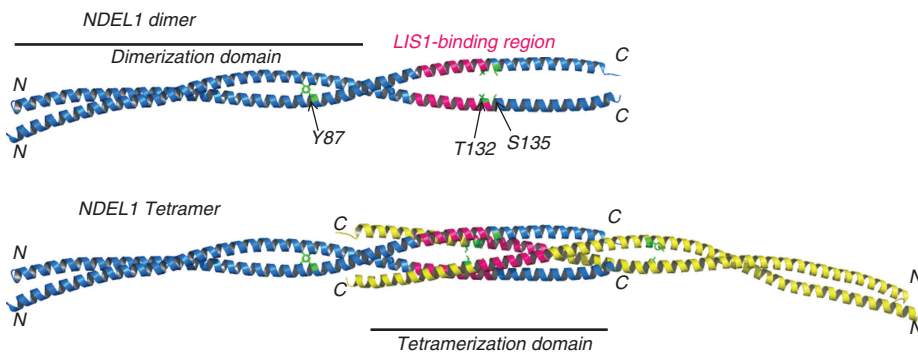


Figure 3 Coiled-coil crystal structure of NDEL1 with known post-translational modifications mapped.

Only three known PTMs map onto the crystal structure of the NDEL1 coiled-coil domain fragment (residues 8–167; PDB ID: 2V71); these side-chains are shown in green and labeled on cartoon representations of the NDEL1 crystal structure, parallel dimer (top panel) and anti-parallel tetramer (bottom panel) (81); N- and C-termini for each chain and regions that facilitate dimerization and tetramerization are indicated. The essential LIS1-binding region on NDEL1 114–133 (96) is also shown (pink). Phosphorylation of NDE1 at T131 (directly equivalent to T132 of NDEL1) reduces its ability to interact with LIS1 (86). It can therefore be hypothesized that phosphorylation of T132 and/or S135 of NDEL1 may have similar effects on LIS1-binding. The Y87 site is highly solvent-exposed on the dimerization domain of coiled-coil structure and is thus readily accessible to kinases.

NDE1 and NDEL1 have different nurture: post-translational modification

Early work revealed that NDEL1 (1, 2) was a phosphoprotein and NDE1 was predicted to contain similar post-translational modifications (PTMs) (3, 4). Since then a number of phosphorylation sites, along with one palmitoylation site, have been experimentally demonstrated to exist in each protein, while a number of others have been suggested with high-throughput proteomic (HTP) experiments using MS (91) (Figure 1; Table 2).

The vast majority of experimentally determined phosphorylation sites reside within unstructured regions of the proteins, predominantly in their C-terminal regions, in particular within the ‘flexible’ proline-rich linker region (87, 88) that connects the coiled-coil domain to the C-terminal α -helix, and others located towards their extreme C-termini. These unstructured regions are likely to be highly solvent-exposed and thus accessible to enzymatic action from protein kinases. Phosphorylation and other PTM sites are usually short motifs that occur within rapidly evolving unstructured regions (92). A number of these phosphorylation sites are not conserved between NDE1 and NDEL1; there are at least 18 sites specific to only one of the proteins (Figure 1). This is in keeping with the notion that rapid evolution of these sites may be linked to gene retention among highly similar duplicated genes such as *NDE1* and *NDEL1*. These differences in PTM present a ready means of effecting functional rewiring (93).

A total of 32 sites have been shown to be post-translationally modified in NDE1 and/or NDEL1 (Table 2; where the equivalent residue is putatively modified in both proteins, this is counted only once). For some of the phosphorylation sites on NDE1 and NDEL1 the kinase or kinases responsible have been identified, while others can be speculated by means of a kinase prediction search of their respective sequences using NetPhosK (94). These results taken together show NDE1 and NDEL1 are substrates for a wide array of kinases (Table 2). It is well known that substrate specificity of protein kinases is highly dependent on the primary amino acid sequence immediately flanking the site of phosphorylation (95); these are classified into basophilic (preference for positively charged amino acids, e.g., PKA and PKC), acidophilic (preference for negatively charged amino acids, e.g., CKI, CKII), proline-directed kinases (preference for proline, e.g., Cdk1, Cdk5, Erk2, GSK-3, MAPK) and other kinases. Both NDE1 and NDEL1 have proline-rich linker regions between their N-terminal

Table 2 Post-translational modification on NDE1 and NDEL1.

NDE1 residue	Known or predicted kinase/enzyme	NDEL1 residue	Known or predicted kinase/enzyme
T7-p	CKI		
S9-p	CKII		
T131-p	PKA (86)	Y87-p	SRC
		T132-p	PKA
		S135-p	–
T191-p	p38MAPK, GSK3	S194-p	CKI, PKC, PKA
		S197-p	CKI
		S198-p	Cdk1, Cdk5 (2, 97)
		S215-p	PKC
S211-p	PKC		
S214-p	–		
T215-p	Cdk1 (41)	T219-p^a	Cdk1, Cdk5, Erk2 (2, 96, 97)
S223-p	RSK, PKC, PKA		
S224-p	–		
T228-p	p38MAPK, Cdk5	S231-p^a	Cdk1, Cdk5 (2, 97)
S231-p	PKC, Cdk1		
S239-p	–		
T240-p	–		
T243-p	Cdk1, Cdk5	S242-p	Cdk1 (96)
T246-p	Cdk1 (41)	T245-p	Erk2 (96)
		S251-p	Aurora-A kinase (97)
C274-s	DHHC2, 3, 7 (54)	C273-s	DHHC2, 3, 7 (54)
Y279-p	–		
S282-p	p38MAPK, Cdk5	S282-p	PKC
S291-p	PKC	T301-p	PKC
S306-p	PKA (88)		
S307-p	–		
T308-p	–		
S309-p	Cdk1	S336-p	Aurora-A and B kinases (101)

Experimentally determined phosphorylation sites (indicated with ‘-p’) from the literature and HTP/MS screens and palmitoylation sites (indicated with ‘-s’) are shown for each protein along with corresponding known kinase/enzyme or NetPhosK v1.0 (94) predicted kinase (stringency prediction threshold=0.5). Where PTM occurs at the equivalent amino acid position in NDE1 and NDEL1 following alignment (Figure 1), these are shown side-by-side. Where the kinase/enzyme is known, these are shown in bold type and their corresponding reference provided; these PTMs are discussed in more detail in the text. All other phosphorylation sites were obtained from the PhosphoSitePlus database (91). ^aPhosphatase known: PP4c acts on T219 (104) and PP2 acts on S231 (100).

coiled-coil domain and predicted C-terminal α -helix, and are significantly enriched for phosphorylation sites therein; it is reasonable to assume that proline-directed kinases are likely to act here. Indeed, both T215 and T246 in NDE1 that possess adjacent proline

residues are phosphorylated by proline-directed kinase Cdk1 (41). Likewise, known sites in NDEL1 – S198, T219, S231, S242 and T245 – are also located adjacent to proline residues and are phosphorylated by Cdk1, Cdk5 and/or Erk2 (2, 96, 97). The C-terminal regions of NDE1 and NDEL1 are highly basic in overall charge (87, 89) and are thus more amenable to basophilic kinases. In support of this, many of the C-terminal sites are suggested to be targets for PKA or PKC, with S306 in NDE1 experimentally verified as a PKA site (88). Future work should aim to experimentally verify the existence of the HTP/MS phosphorylation sites using additional techniques such as amino acid sequencing, phospho-specific antibodies, site-directed mutagenesis, dominant-negative constructs etc. and confirm the remaining kinases that act on NDE1 and NDEL1 based upon this exercise.

Structural landscape of post-translational modification: implications for NDE1 and NDEL1 function

In order to discuss in detail the potential and known effects of NDE1 and NDEL1 PTM, each structural domain or region of the proteins will now be considered in turn. Schematics displaying the overall PTM landscape on NDE1 and NDEL1 structure along with known protein-protein interaction sites are shown in Figure 4.

N-terminal coiled-coil domain

The N-terminal region of NDEL1 that is involved in the formation of oligomers through coiled-coil interactions

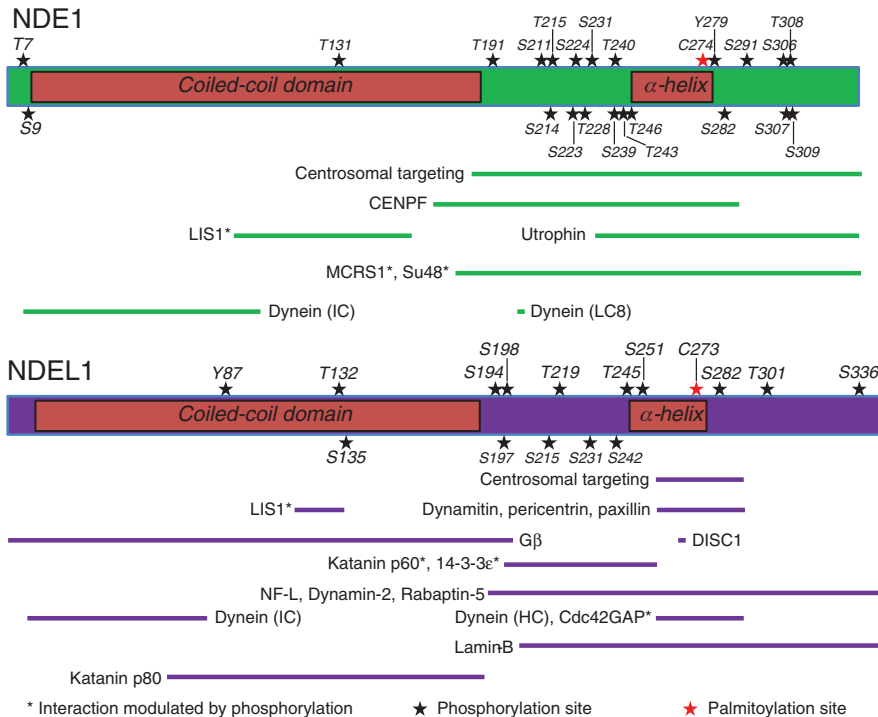


Figure 4 Domain architecture, post-translational modification and binding/functional regions of NDE1 and NDEL1.

Schematics of NDE1 (top panel) and NDEL1 (bottom panel) drawn to scale depicting the N-terminal coiled-coil domain and the predicted C-terminal α -helix (labeled); the central flexible linker region connects these two structured regions, while another unstructured region lies at the extreme C-terminus of the proteins. Numerous PTMs (stars) reside within delineated protein-protein interaction sites, sub-cellular localization regions, or oligomerization domains with the vast majority located within predicted unstructured regions of the proteins. Minimal essential protein-binding sites from the literature are shown drawn to scale (NDE1: green bars; NDEL1: purple bars). References for each NDE1 interaction site are as follows: Centromere protein F (CENPF)/Mitosis/LEK1 (112); LIS1 (4); Utrophin (66); MCRS1/p78 (99); Su48/DBZ/ZNF365 (98); Dynein IC (89); Dynein (LC8) (49). References for each NDEL1 interaction site are as follows: Centromere protein F (CENPF)/Mitosis/LEK1 (112); LIS1 (96); Dynamitin (50); Pericentrin/Kendrin (113); Paxillin (114); G-protein β ($G\beta$) (115); DISC1 (28); Katanin p60 and p80 (68); 14-3-3 ϵ (100); Neurofilament (NF-L) (72); Dynamin-2 (75); Rabaptin-5 (78); Dynein intermediate chain (IC) (89); Dynein heavy chain (HC) (1); Cdc42GAP (74); Lamin-B (63). Note: the palmitoylation site C273 (red star) on NDEL1 is also the site of endooligopeptidase activity (77). In the majority of cases, these sites have only been investigated for one of NDE1 or NDEL1; it is likely that some of them will be conserved between the two proteins. The effect of PTMs should be tested on protein conformation, stability, oligomerization, modulation of protein-protein interaction and cellular trafficking/targeting.

remains the only region of the two proteins to be experimentally determined by crystallography (81), allowing the locations of the known phosphorylation sites in this region to be mapped on its three-dimensional structure (Figure 3).

Only three phosphorylation sites are known to reside within the N-terminal 190-amino acid coiled-coil-containing region for each of NDE1 and NDEL1 to date. NDE1 was shown to be phosphorylated by PKA at T131 with both DISC1 and phosphodiesterase 4 (PDE4), interaction partners of NDE1, able to modulate this post-translational event (86). Phosphorylation of this site causes increased interaction of NDE1 with NDEL1 in COS7 cells, but decreased interaction with LIS1 (86). Given that this region of NDE1 and NDEL1 governs their oligomerization/tetramerization state (81, 87) and that NDE1 and NDEL1 are capable of interacting in the same manner as NDE1 and NDEL1 homomeric species (87), one possibility is that this site may modulate the dimeric vs. tetrameric state of NDE1 generally, with the observed effect on hetero-oligomerization of NDE1-NDEL1 representing a special case of this. The HTP/MS-derived phosphorylation site at S135 of NDEL1 would likewise be predicted to impact on similar processes given its similar characteristics to T131, such as its location within an incomplete heptad repeat within the tetramerization domain (81, 86) and proximity to the LIS1-binding region (Figure 3). Potentially as a consequence of this, expression of NDE1 with a T131 phosphomimic mutant leads to decreased neurite outgrowth in a NS-1 cell assay (86). Based on amino acid sequence identity, NDEL1 would be predicted to be phosphorylated at its equivalent site, T132, however two studies have been unsuccessful in demonstrating this (86, 90), although it has been detected in a single HTP/MS screen (91). An antibody which could recognize both T131-phosphorylated NDE1 and putative T132-phosphorylated NDEL1 species, showed such species to be present at post-synaptic densities and cortical primary axons of mice, and to remain at the spindle poles of COS7 cells throughout cell mitosis, in contrast to non-phosphorylated species (86).

Central flexible linker region

As previously noted, the unstructured region between the N-terminal coiled-coil and predicted C-terminal α -helix of both NDE1 and NDEL1 are highly phosphorylated, with NDEL1 being a substrate for Cdk5 at S198, T219 and S231 (2). Cdk1 has been variously reported to either phosphorylate these sites or T219, S242 and T245 (96, 97), while Erk2 acts at T219 and T245 (96). Equivalent phosphorylation of

NDE1 is less well studied; however, it is known to be a substrate for Cdk1 (98), which acts on T215, T246 and at least one additional undefined site (41). Cross-talk between these kinases also occurs, with use of a dominant-negative version of Cdk5 preventing Cdk1 from phosphorylating S231 of NDEL1, while the reverse was not true when a dominant-negative form of Cdk1 was used (62).

Several studies have looked at the expression and function of NDEL1 species in which five putative phosphorylation sites in this region (S198, T219, S231, S242 and T245), including all known Cdk1, Cdk5 and Erk2 sites were substituted to make either phosphodead alanine or valine residues, or to mimic phosphorylation with glutamic acid residues. NDEL1 carrying phosphodead mutations of all five of these residues are found prominently at the centrosome during interphase, as was wild-type NDEL1; in contrast, NDEL1 with five phosphomimic residues was only weakly detectable there (96). Similarly, the phosphomimic failed to transfer to the spindle poles during telophase in the manner of wild-type NDEL1; by contrast, the phosphodead mutant was prominent at the spindle from metaphase (96). Phosphorylation of NDEL1 therefore appears to block its spindle localization until the appropriate part of the cell cycle. Similar results were also seen when five putative phosphorylation sites in this region of NDE1 (T191, T215, T228, T243 and T246), plus one immediately C-terminal of its predicted α -helix (S282), were all mutated to phosphodead alanine or valine residues (98). Dramatically, knock-out of one such site on NDE1, T246, arrested mitotic HEK293T cells at the G2/M phase boundary (41).

Phosphorylation of residues in the central flexible linker region have been shown to modulate the interactions with other centrosomal proteins, specifically of NDE1 with the centrosomal protein Su48 (also known as DBZ or ZNF365) (98) and mitotic protein Microspherule protein 1 (MCRS1; also known as p78) (99), as well as the interactions of NDEL1 with the microtubule severing protein katanin (68) and 14-3-3 ϵ (100) (Figure 4); although other phosphorylation events have been reported to have a greater impact upon 14-3-3 ϵ interaction (101). Mock phosphorylation of NDEL1 at these residues also leads to increased activation of Cdc42GAP (74). Of particular interest though is the effect of these phosphorylation events on LIS1 and dynein. Application of either Cdk1 or Cdk5 to NDEL1 led to an increase in its interaction with LIS1, while causing dissociation of LIS1 to dynein (62). Successful dissociation of LIS1 is vital in the process by which dynein moves along microtubules with its cargo (102) and the importance of NDEL1 phosphorylation in this is seemingly demonstrated by the fact that expression of

mutant NDEL1 lacking the five putative phosphorylation sites, or expression of a dominant-negative Cdk5 mutant, each effectively abolished dynein-related organelle transport along the axons of rat neurons (103). It is therefore possible that Cdk5 as well as potentially Cdk1 and Erk2 phosphorylation of NDEL1 may modulate dynein activity through altering LIS1-dynein interaction. Mutation of NDEL1 residues T219 and T245 was also shown to block NDEL1-dynein interactions (89). Other dynein-related effects of phosphorylation sites in this region are known; they are required to increase prophase nuclear envelope invagination in response to Cdk1 or Cdk5 (62), while their mutation inhibits cell migration during scratch-wound assays (74).

It has recently been shown that the C-terminal α -helix of NDE1 and NDEL1 can interact directly with its N-terminal coiled-coil domain (87) facilitated by this central proline-rich unstructured region that permits a bent-back conformation. It is therefore reasonable to suggest that many of the effects attributed to phosphorylation of residues in this region could be a consequence of regulation of this conformational process. For example, the effects on dynein function may occur because altered phosphorylation of this region affects the ability of NDE1 and NDEL1 to bring their distinct N- and C-terminal dynein binding domains (60, 89) into close physical proximity.

At least two separate phosphatases are involved in the removal of phosphate groups from this region of NDEL1, with protein phosphatase 4 catalytic subunit (PP4c) acting on T219 (104) and protein phosphatase 2 (PP2) acting on S231 (100) (Table 2). Interestingly, the former phosphatase is present at the centrosome at all times except during mitosis, suggesting that it may be involved in temporal regulation of NDEL1 function there (104).

Predicted C-terminal α -helix

The predicted α -helix of NDEL1 (87, 88) contains the DISC1 binding site (28) and overlaps with the ‘minimally’ defined binding regions of the dynein heavy chain (1) (Figure 4). The most investigated PTM in this region, is phosphorylation of NDEL1 by Aurora-A kinase at S251 during prophase, with expression of a NDEL1-S251E phosphomimic mutant being sufficient to rescue mitotic entry defects caused by Aurora-A knockdown (97). At the end of prophase, these phosphorylated NDEL1 species rapidly disappear, seemingly due to ubiquitination followed by degradation by the proteasome (97). This phosphorylation event also occurs in post-mitotic neurons, where phosphorylation of NDEL1 at S251 occurs as a result of Aurora-A-kinase activation by

PKC- ζ at connections of the neuronal soma with neurites (71). Disruption of the function of any of PKC- ζ , Aurora-A kinase or NDEL1, variously by use of pseudo-substrates, kinase-deficient mutants or RNAi, led to decreased neurite extension in dorsal root ganglia neurons (71). Expression of a NDEL1-S251E phosphomimic mutant was also able to rescue migration defects in granular neurons caused by either conditional knockdown of endogenous NDEL1 or inhibition of Aurora-A kinase (105). The mechanism by which S251 phosphorylation causes these effects is unclear, although one possibility emerges from the fact that, unlike wild-type NDEL1, an S251A phosphodead mutant was not able to interact with dynein (89).

Additionally, NDEL1 is known to interact with 14-3-3 ϵ as well as potentially 14-3-3 α/β , γ , τ and ζ (100). S251 of NDEL1 is a good match for the canonical phosphorylation-dependent binding site of 14-3-3 proteins and indeed mutation of S251 to alanine reduces NDEL1 interaction with 14-3-3 (101). Additionally, interaction between recombinant forms of the two proteins is increased following Aurora-A kinase treatment (101).

NDEL1 can also be palmitoylated within this predicted helix at C273 by three enzymes, DHHC2, 3 and 7, causing a reduction in interaction between NDEL1 and dynein and an increase in NDEL1 at neurite tips, indicating reduced retrograde transport by dynein (54). Other evidence that NDEL1 palmitoylation limits dynein function can be seen through its impaired ability to transport proteins, short microtubules and Golgi vesicles following co-transfection of DHHC and wild-type NDEL1, but not when a DHHC and a NDEL1-C273S palmitoylation-resistant mutant or NDEL1 alone were used (54). It has also been reported that expression of NDEL1 with a C273A mutation inhibits the growth of neurites of PC12, in contrast to wild-type NDEL1 which increases it, although a specific role of palmitoylation in this process has not been established (106). Similarly, wild-type, but not C273A, NDEL1 can rescue neurite outgrowth defects caused by knockdown of endogenous NDEL1 (106). NDE1 can also be palmitoylated at its equivalent site, C274, by the same DHHC enzymes as NDEL1; however, strikingly, palmitoylation of NDE1 had no effect on its interaction with dynein or any of the other functional tests of NDEL1 palmitoylation, indicating a fundamental difference in the function of NDE1 and NDEL1 (54).

C273 is also the catalytic residue for the oligopeptidase activity of NDEL1 (77), although it remains unclear whether this is blocked by palmitoylation, or whether the effect on neurite outgrowth ascribed to NDEL1 species in which C273 has been mutated is due to altered enzymatic activity, altered interaction with dynein or a combination of the two. The possibility exists that this palmitoylation

event may act as a switch between these two, seemingly distinct, functions of the NDEL1 protein. Although the putative catalytic cysteine residue is also present in NDE1, no study to date has investigated whether it also possesses a similar oligopeptidase activity.

Extreme C-terminal unstructured region

The extreme C-terminal region of NDE1 and NDEL1 is known to interact with a number of different protein binding partners, and it is therefore likely that the PTMs within this region influence NDE1 and NDEL1 function at least in part through regulation of these interactions. Of these, NDE1 is known to be phosphorylated by PKA at S306 (88), in a DISC1- and PDE4-modulated process (86), while the equivalent residue in NDEL1 is non-phosphorylatable (Figure 1). The function of this site remains unknown; however, NS-1 cells expressing NDE1-S306D phosphomimic mutants display an increase in neurite number compared to those expressing phosphodead S306A mutants (86). It is notable that proteomics screening implies S306 is part of a cluster of four directly concurrent phosphorylated residues, three of which are specific to NDE1 (Figure 1). The functional significance of this cluster remains to be determined.

Additionally, NDEL1 has a serine at residue 336 which can be phosphorylated by Aurora-A and -B kinases, creating a 14-3-3 binding site (101). Mutation of this site to alanine has a greater effect on 14-3-3 interaction than similar mutation of S251 described earlier, with mutation of both abolishing the majority of interaction (101). Notably, however, this site exists only within a specific isoform of NDEL1 and because both NDE1 and NDEL1 are known to exist as multiple splice variants, many of which vary only in their extreme C-termini (33) it can therefore be speculated that one purpose of the multiple splice variants is to introduce alternative PTM regulation at the extreme C-terminus of a specific subset of NDE1/NDEL1 molecules *in vivo*, and potentially ones with unique patterns of temporal or spatial expression with the organism.

Conclusions

Since their first description, NDE1 and NDEL1 have emerged as key players in mitosis and neurodevelopment with overlapping yet distinct functional profiles. While they share high sequence similarity and have been shown to adopt similar structure and oligomeric states, they are

clearly differentially regulated post-translationally. A mass of PTM data has emerged for the two proteins over the past decade and recent HTP data via tandem MS will continue to inform on regulatory mechanisms of protein function. Notwithstanding the expert manually curated effort of the PhosphoSitePlus database (91) to catalogue experimentally determined PTM sites, it should be borne in mind there could be some data quality issues for assignments; false positives and/or incorrect assignments to either NDE1 or NDEL1, given their high sequence identity, is a possibility, especially for some of the sites identified purely via MS/HTP-discovery, whose assignments are probabilistic by nature. Nonetheless, nine out of ten sites verified in the primary literature for NDE1 and NDEL1 were seen in the MS-only assignments in the database suggesting a high true positive rate. Even for the one site T131 in NDE1, not discovered by MS, the equivalent NDEL1 site (T132) was indeed observed. Additionally, for each protein >50% of sites assigned only using the MS/HTP criteria had five or more references corresponding to each site in the database, indicating a high potential for these sites to be true positives too. Conversely, it should also be noted that there could be a failure to detect some of the phosphorylation sites in a protein sample because of the inherent sensitivity of the methodology whereby the phosphate groups can be lost during sample preparation and analysis. Hence, failure to detect phosphorylation should not necessarily be considered as evidence that the site is not actually phosphorylated under certain relevant conditions (107).

A major issue in clarifying the relationship between NDE1 and NDEL1 remains the relative lack of directly comparable information between them. For example, while there are some proteins confirmed to be interaction partners of both NDE1 and NDEL1, there are a greater number which are known to interact with NDEL1, but for which comparable interaction studies with NDE1 have not yet been performed (Figure 5). Some examples of protein interaction partners proven to bind NDE1, but not yet tested for NDEL1, also exist.

From the information currently available, however, it is possible to begin to speculate on the fundamental differences between the NDE1 and NDEL1 proteins which have warranted their conserved existence throughout vertebrate evolution. Functionally, the proteins appear to be highly similar, but with potential differences in dynein binding (49) and mitotic function (58). By contrast, their post-translational regulation is distinct; this suggests that, due to the fundamental roles the two proteins play in neuronal function, the existence of two distinct 'Nude-derived' proteins allows a tighter level of regulation of

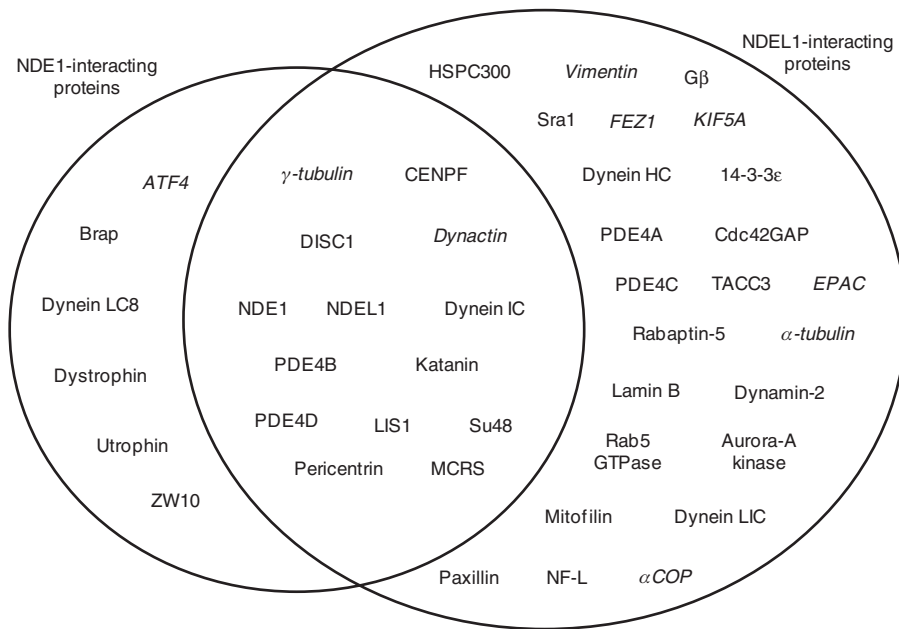


Figure 5 Protein interaction partners of NDE1 and NDEL1.

Proteins in the left circle interact with NDE1, those in the right circle with NDEL1. Proteins in the intersecting region are known to interact with both proteins. In the majority of instances where a protein is listed as interacting with only one of NDE1/NDEL1, the reciprocal experiment to test for interaction with the other of NDEL1/NDE1 has not been published to the best of our knowledge. Proteins listed in italics are known to exist in a complex with NDE1/NDEL1 but do not, or have not yet been shown to, directly bind to it. Data are taken from the following papers (1–5, 27–29, 31–33, 48, 50, 53, 57, 58, 63, 65, 66, 68, 72, 74–76, 78, 80, 88, 90, 96–98, 100, 101, 113–121). In some cases the essential binding regions for these interaction partners have been delineated on NDE1 or NDEL1; these are shown schematically in Figure 4.

their roles. It is also noteworthy that while *Ndel1* knock-out mice are not viable (51), *Nde1* knock-out mice survive to birth (57). We therefore hypothesize that while NDEL1 plays the “core” role, conserved across evolution of NDE1/NDEL1 orthologs, NDE1 can act as an ancillary version, providing additional or perhaps slightly modified versions of the common NDE1/NDEL1 functions where necessary under a different system of regulation. Such differences are likely to be both temporal, with NDE1 having a more specialized pattern of expression during early neurodevelopment, and spatial, with NDEL1 known to have both cortical and hippocampal developmental functions (51), while NDE1 has mainly been described as affecting the cortex (57). An alternative theory to explain the mouse viability, i.e., *Ndel1* has a specific essential function in the early blastocyst, must also be considered. The former model would, however, be consistent with the relatively higher conservation of NDEL1 throughout evolution, compared to the more recent, faster evolution of NDE1 (54) (Figure 1; Table 1). This would also be consistent with the apparent role of *NDE1* CNVs in mental health and other neurodevelopmental conditions: while gross disruption of the more ‘essential’ NDEL1 protein would be critical and perhaps incompatible with life, disruption of NDE1 causes

more subtle effects by comparison, notwithstanding the gross microcephaly phenotypes associated with complete loss of NDE1 function. The issue of exactly when and where NDE1 and NDEL1 perform their functions is further complicated by their ability to form hetero-oligomers (29, 33, 87), leading to the possibility of unique functions of a mixed NDE1-NDEL1 species, in addition to those performed by each protein alone. Teasing apart this relationship remains an important avenue for future research; research that will be of significant importance in understanding their shared and specific roles in psychiatric disorders and neurodevelopment.

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