COMMENTARY

Assembly of NFL and desmin intermediate filaments: Headed in the right direction

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Intermediate filaments (IFs), together with actin filaments and microtubules, form the cytoskeleton—a critical structural component of all cells. Humans express 73 unique IF proteins that associate as obligate homo- or heterodimers. IF dimers assemble into tetramers, which are the building blocks of the higher-order cytoskeletal and nucleoskeletal structures visualized in vitro and in cells (1). Cytoplasmic IFs are functionally suited to the needs of each cell and tissue type. For example, keratins provide mechanical resilience to the epidermis (2), whereas the light, medium, and heavy neurofilament proteins (NFL, NFM, and NFH) simultaneously provide axons with the stability and flexibility needed for proper neuronal function (3). In the past several decades, significant advancements have been made in the basic biology and disease involvement of IFs, but insights into the molecular structures are lacking for most IF proteins (4). In PNAS, Zhou et al. (5) address the hypothesis that interactions between N-terminal low complexity domains (LCDs) on IF proteins, shown previously to undergo phase separation (6), could provide structural insights into how mature filaments are formed.

All IF proteins have three domains: N-terminal "head," C-terminal "tail," and central coiled-coil "rod" (Fig. 1) (1). The rod domains, which are highly conserved, form the dimer interface, while the head and tail are variable regions composed of few overrepresented amino acids (i.e., of low complexity) (5, 6). The present work builds upon previous observations that the aliphatic alcohol 1,6-hexanediol, which disrupts nonmembrane-bound structures and phase-separated liquid-like droplets, is able to rapidly disassemble vimentin and keratin IFs without affecting the organization of actin or microtubules in cells (6). This ultimately helped reveal that the head (but not the tail) domains of several human IFs, including vimentin, peripherin, α-internexin, and neurofilaments, form gel-like condensates composed of uniform polymers that are labile to disassembly (6).

The authors took advantage of this system to ask whether NFL head domains within these phase-separated assemblies are able to form specific interactions with native proteins from normal mouse brain lysates. Interestingly, the top protein that was captured upon incubation of the mouse brain lysates with NFL head domain hydrogels was the NFL protein endogenous to the mouse brain (based on the presence of peptides corresponding to all three domains). Specificity of self-association was confirmed by comparing to hydrogels composed of LCDs of several other non-IF proteins, which did not capture mouse brain NFL. Furthermore, specificity in NFL head–head domain interactions was demonstrated by incubating NFL head domain hydrogels with recombinant full-length NFL or head/rod/tail segments separately and in combination with the adjacent domain. Only constructs containing the NFL head or NFL head + rod domains associated with NFL head domain hydrogels, confirming the requirement for the presence of the NFL head LCD. Similar reconstitution experiments were performed with desmin, which behaved in the same way as NFL.

Having established specificities of self-association with test hydrogels, the authors next addressed whether the polymers exhibited secondary structure. To that end, two-dimensional solid-state NMR (ssNMR) spectra of the NFL and desmin head domain polymers revealed a predominance of β-strand secondary structure. While the cross-peaks were not sufficiently resolved to allow determination of the precise level of order, in-register parallel β-sheets, as what is seen in most amyloid-like assemblies, were ruled out based on calculated ^{13}C–^{13}C distances. The critical next question was whether the structures observed with the NFL head and desmin head domain-only assemblies are biologically significant. This was tested by comparing the ssNMR spectra of head domain-only assemblies to the spectra of fully assembled mature filaments containing all three domains. The latter was accomplished by combining intein chemistry and isotopically labeled amino acids to prepare full-length NFL and desmin assemblies. Out of three potential outcomes, two would have argued against biological

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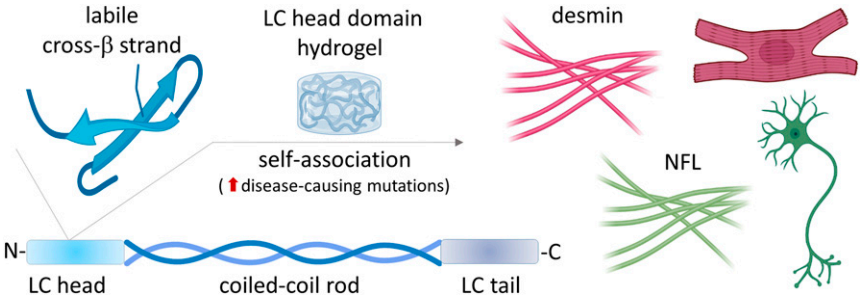

Fig. 1. The LC head domains on neurofilament light (NFL) and desmin form labile cross–β-strands that promote self-association and filament assembly. All 73 human IF proteins share a common domain structure, which consists of a central α-helical rod domain (forming the dimer interface) flanked by N-terminal head and C-terminal tail, which are low complexity (LC) domains. While previously thought to function in the absence of structural order, Zhou et al. (5) show that the N-terminal head domains form labile cross–β-strands that promote self-association to facilitate assembly of desmin and NFL in vitro. Note that the single generic β-strand depicted in the illustration is not representative of structure elucidated from this work. Multiple disease-causing mutations affecting the LC head domains of desmin and NFL significantly enhance self-association, providing insight on IF proteostasis mechanisms. Combined with previous studies showing that the LC head domains drive phase separation of IFs (forming hydrogels composed of amyloid-like polymers) and that IFs associate directly with RNA and RNA-binding proteins, these findings suggest potential involvement of NFL and desmin in the localization of ribonucleoprotein particles in myocytes and neurons. Image created using Biorender under an academic license.

significance: if structure was absent or structure was present but did not correspond to what was observed with the head domain-only assemblies. Neither of those two outcomes materialized; the authors showed, both qualitatively and quantitatively, that the spectra obtained from the head domains within the NFL and desmin full-length assemblies were the same as the head domain-only assemblies. Furthermore, the spectra of the full-length proteins, when assayed at different temperatures, provided evidence that the head domains contain both unstructured and structured segments, but there was no interconversion between the two states upon temperature alteration.

Phosphorylation of the IF head domains is known to promote filament disassembly, and approximately one-third of the amino acid contents of the NFL and desmin head domains are accounted for by serine residues. In further probing the dynamics of the LCD phase-separated assemblies, the authors demonstrated that phosphorylation by protein kinase A (PKA; a physiological kinase for many IFs) released NFL and desmin head domains from the hydrogels. This is in line with the known roles of phosphorylation in promoting filament disassembly (7) and demonstrates that the PKA sites are accessible in the context of hydrogel-bound NFL and desmin assemblies.

The IF cytoskeleton is important for homeostasis and critical for allostasis—the ability to regain homeostasis following exposure to stress (8, 9). Paramount to these functions are the adaptability and flexibility of IF structures to meet diverse cellular demands. This is thought to be achieved via protein synthesis-independent turnover, or cycling, of the IF network facilitated by the motile behavior of filament subunits in cells (10, 11). Naturally occurring mutations in IF genes that interfere with the dynamic behavior of the filament networks cause both tissue-specific and systemic human diseases, including progressive and fatal neuropathies, myopathies, skin fragility disorders, metabolic dysfunctions, and premature aging syndromes, among many others (12). Abnormal filaments and prominent intracellular IF aggregates are common to most of these diseases, resulting from direct gene mutations, but in other cases they can arise as a result of chronic unresolved stress (13–18). In parallel studies using recombinant human NFL and desmin, the authors show that self-association facilitated by the respective LC head domains is significantly enhanced in the context of Charcot–Marie–Tooth-causing mutations in NFL (residues Pro8 and Pro22) and myopathy-causing mutations on desmin (Ser residues at positions 2, 7, 12, 13, and 46). Therefore, aberrant LC head domain self-association may be involved in the pathologic accumulation of mutant NFL and desmin in these diseases.

In conclusion, Zhou et al. demonstrate that the N-terminal LCDs of NFL and desmin form labile cross-β strands that promote self-association and facilitate filament assembly in vitro (Fig. 1) (5). The findings challenge the prevailing view that, while critical for mature filament assembly, the N-terminal domains on IFs are structurally amorphous. Beyond providing molecular-level views of the assembly of NFL and desmin, these findings open exciting new possibilities to understand how dysfunctional IFs in neurons (19) and myocytes (14) cause human diseases. Together with previous discoveries by the McKnight (6) and Eisenberg laboratories (20), the present findings also lend support to the hypothesis that different IFs, through their ability to undergo LCD-mediated phase separation, may function to localize RNA granules in cells. Specifically, it was shown previously that vimentin filaments bind the fused in sarcoma (FUS) RNA-binding protein (6) and that epithelial keratins contain aromatic-rich, kinked segments (LARKS), which are thought to facilitate nonmembrane-invested LCD assemblies of many different types of proteins (20). In addition, both desmin and vimentin have been identified as RNA-binding proteins in cardiomyocytes using global profiling studies (21). Further validating and interrogating these interactions in cellular and animal models will illuminate how structure dictates function and provide opportunities for mechanism-based targeted therapies of IF diseases.

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