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New Concepts

Dynein Light Chain LC8 Is a Dimerization Hub Essential in Diverse Protein Networks†

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ABSTRACT: The operations within a living cell depend on the collective activity of networks of proteins, sometimes termed "interactomes". Within these networks, most proteins interact with few partners, while a small proportion of proteins, called hubs, participate in a large number of interactions and play a central role in organizing these interactomes. LC8 was first discovered as an essential component of the microtubule-based molecular motor dynein and as such is involved in fundamental processes, including retrograde vesicular trafficking, ciliary/flagellar motility, and cell division. More recently, evidence has accumulated that LC8 also interacts with proteins that are not clearly connected with dynein or microtubule-based transport, including some with roles in apoptosis, viral pathogenesis, enzyme regulation, and kidney development. Here, we introduce the idea that LC8 is a hub protein essential in diverse protein networks, and its function as a dynein light chain is but one of many. We further propose that the crucial regulatory roles of LC8 in various systems are due to its ability to promote dimerization of partially disordered proteins.

Cytoplasmic dynein is a minus end microtubule-based molecular motor that is implicated in a variety of processes, including mitotic spindle assembly and orientation, chromosome segregation (*1*), intracellular trafficking of vesicles and mRNA (*2*), establishment of cell polarity (*3*), and development and maintenance of neurons (*4*). LC8 is a highly conserved 10 kDa component of the dynein complex. The human sequence is identical to those of other mammals and 94% identical to that of *Drosophila*. Its crucial role has been demonstrated in several organisms, including *Drosophila*, where null mutations result in embryonic lethality (*5*).

LC8 is assembled in the dynein complex through direct interaction with the N-terminal domain of the dynein intermediate chain (IC) (*6*, *7*). LC8 also associates with a wide variety of proteins, including neuronal nitric oxide synthase (nNOS), myosin V, the proapoptotic factor Bim, transcriptional factors Swallow and Trps1, viral proteins, and p53 binding protein 1 (*8*−*14*). Because these proteins are found to be associated with LC8, they have been presumed to be dynein cargo, and LC8 has been proposed to function as a versatile adapter that links cargo proteins to the dynein motor (*8*, *15*). However, a significant proportion of LC8 is not associated with the dynein complex (*16*), raising the suggestion that LC8 plays roles unrelated to dynein (*17*).

NMR and crystallographic studies reveal that when bound to the LC8 dimer, IC, Bim, nNOS, and Swallow occupy the same binding grooves at the dimer interface and form a sixth β-strand of the LC8 antiparallel β-sheet (Figure 1) (*15*, *18*, *19*). Binding of IC in the same grooves as these putative cargoes raises the question of how LC8 simultaneously assembles with dynein and transports cargo. One proposal

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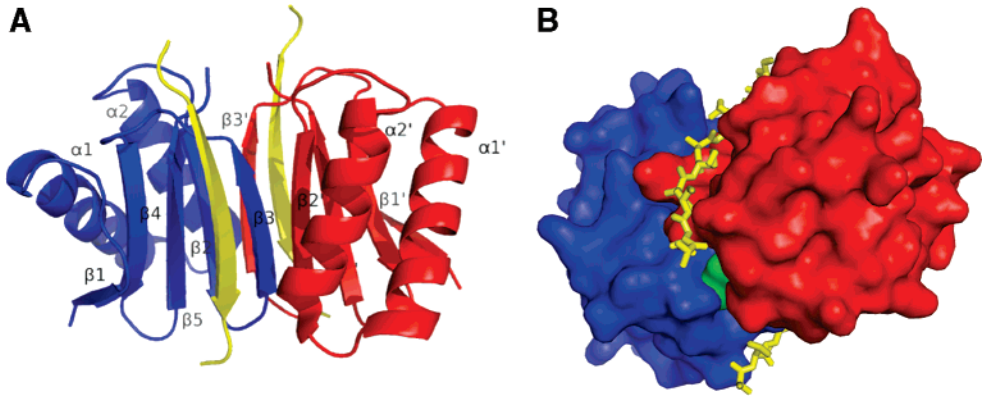

FIGURE 1: LC8 bound to Swallow peptide. (A) Ribbon diagram of LC8 dimer (blue and red) with the peptide colored yellow. Elements of LC8 secondary structure are labeled. (B) Surface representation of LC8 dimer with the peptides as a yellow stick model at the dimer interface. Phosphorylation of Ser 88 (green) results in dimer dissociation (*20*). In both images, the PDB 2P1K crystal structure (*19*) is visualized by PyMol (*42*).

is formation of a ternary LC8−IC−cargo complex, where one groove on LC8 binds dynein IC and the other binds cargo (*15*). Recent structural and binding studies (*19*, *20*) show that in the case of Swallow, which has long been considered a cargo protein, the formation of such a ternary complex is questionable. While both IC and Swallow have similar overall structures of their consensus residues when bound to LC8 (*19*), association of Swallow in vitro is much tighter than that of IC (*20*), and Swallow would therefore be expected to outcompete IC at both LC8 dimer binding grooves. Consequently, it cannot be assumed that in vivo LC8 simultaneously binds IC and Swallow, and if they are not bound at the same time, Swallow is not likely to be linked to dynein via LC8 for transport.

In protein−protein interaction networks, most proteins interact with few partners, while a small proportion termed "hubs" interact with many diverse partners and are essential for maintenance of the global network structure and for regulation of various processes in the cell (*21*). Sequence analyses of hub proteins and their interacting partners shows that many of these proteins have regions of intrinsic disorder, which led to the proposal that intrinsic disorder in one or both partners enables them to interact with numerous targets (*22*, *23*).

Here we propose that LC8 is an ordered hub protein that interacts with diverse proteins containing regions or domains of intrinsic disorder and mediates their increased level of structural organization and dimerization. This activity as a dimerization hub that facilitates specific association of partially disordered proteins would unite the diverse roles LC8 apparently plays with disparate partners in numerous cellular systems and explain in broad terms how LC8 functions both in dynein and in unrelated complexes.

LC8 as a Dimerization Engine in Dynein. LC8 is an integral component of the dynein complex, present as dimer in 1:1 stoichiometry with two copies of IC. In vitro studies with a 300-amino acid N-terminal domain of IC, which is intrinsically disordered and monomeric, show a gain in the content of secondary and compact structure of IC upon LC8 binding (*6*, *24*). Increased helical structure content in this IC domain is indicated by an 8% increase in the CD-detected helix content of IC (*24*), which corresponds to a transition of 24 residues to helix. A segment of IC which is 100 residues from the LC8 binding site and predicted to form a coiled coil is necessary for the increase in helical structure content and is shown by NMR to have helical propensity (*25*). On the basis of these observations, we have proposed that LC8 is required for efficient dimerization of IC (*24*, *25*) as diagrammed in Figure 2, where binding of two monomers of an IC segment to LC8 dimer increases the effective local concentration of monomeric IC and drives its dimerization by formation of a helical coiled coil in residues 220−240, which are distant from IC residues 123−138 that directly bind LC8. The model does not suggest full ordering of IC upon LC8 binding, in keeping with the moderate increase in helix content. Also, a minor population of dimer IC in the absence of light chains (*26*) is not excluded by this model.

LC8 Is Not Primarily an Adaptor Protein for Dynein Cargo. The role of LC8 as a cargo adaptor linking proteins to dynein for transport along microtubules has been suggested for several binding partners, but a clear relation between the interaction of LC8 and active microtubule-based transport was demonstrated for only a few. The interaction of LC8 with Egalitarian (Egl), a protein essential for establishing and maintaining polarity during oogenesis and embryogenesis in *Drosophila*, is one such example (*27*). Mutations that disrupt binding between Egl and LC8 result in failure of accumulation of oocyte-specific markers, which depends on the association with a microtubule-based motor. Another interaction is with Dazl which is required for mRNA localization in male germ cell development in mammals, the distribution of which is microtubule-dependent (*28*). LC8 was also proposed to link the tumor suppressor binding protein (p53BP1) to dynein to actively transport p53 which undergoes dynein-dependent nuclear transportation in response to DNA damage (*8*). With Swallow, LC8 binding is required for dynein-dependent transport of bicoid mRNA to the oocyte anterior cortex (*11*).

Except for Dazl, which associates with the microtubule network via the dynein−dynactin complex through LC8 (*28*), no experiments to date show definitively that LC8 is a component of the dynein complex in the various interactions listed above. On the contrary, Egl clearly associates with the dynein complex *without* LC8 (*27*). With Swallow, LC8 is not sufficient for its localization along microtubules since even in the presence of LC8, mutations in the C-terminal region of Swallow block its anterior localization (*11*),

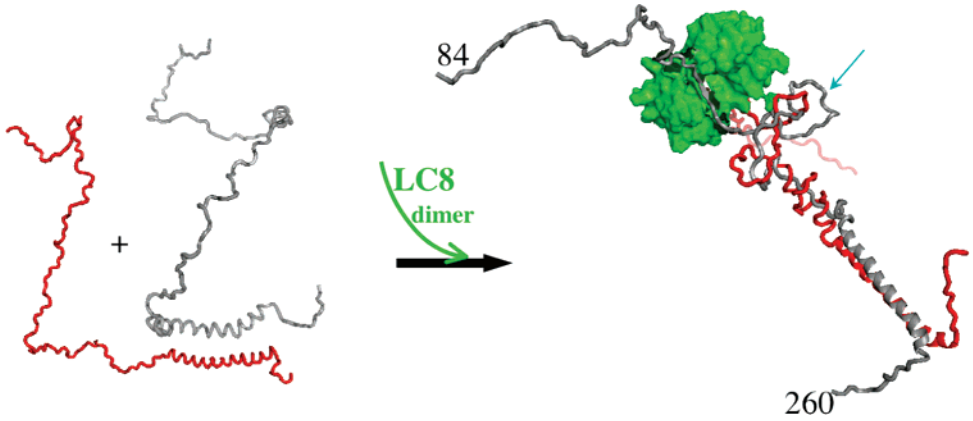

FIGURE 2: Model for dimerization and structural organization of IC associated with LC8 dimer binding. On the left side of the diagram, two molecules of IC (IC84−260) are shown as primarily disordered monomers (gray and red) with a nascent helix corresponding to a predicted coiled-coil domain. Upon binding of LC8 dimer (green arrow and surface model), two IC subunits are brought into the proximity of each other, which favors IC dimerization. Increased IC helical structure content is depicted on the right side of the diagram as a dimeric coiled coil. A linker domain comprised of IC residues between the LC8 binding site and the putative coiled coil, at which an arrow points, is drawn as somewhat collapsed; however, the CD data do not indicate that these residues are ordered, and apparently they remain flexible. This diagram is adapted from Figure 10 of ref *25*.

suggesting that the C-terminal domain and LC8 binding are both responsible for its localization.

Interaction of LC8 with the rabies virus phosphoprotein is essential for virus pathogenesis and was proposed to link rabies virus to dynein for axonal transport through neuron cells (*14*). However, removal of the LC8-binding domain from rabies virus protein decreases the extent of primary transcription but does not affect dynein-dependent viral entry into the cell, clearly demonstrating that LC8 interaction with the phosphoprotein is independent of its role as a dynein subunit (*29*). Similarly, in the interaction with Nup159 of the nuclear pore complex in yeast (*30*), LC8 functions as a nucleoporin which coclusters with the nuclear pore complex while the dynein heavy chain is not detected at the nuclear envelope. Furthermore, deletion of the gene encoding dynein heavy chain does not retard growth like deletion of the LC8 gene does, demonstrating that LC8 is essential for cell growth in a manner independent of the dynein motor complex.

LC8 as a Dimerization Engine for Non-Dynein Partner Proteins. The dimeric structure of LC8 with dual symmetric binding sites, along with the presence of helical, coiled-coil, or other dimerization domains in the binding partners, could be the underlying features that account for the ability of LC8 to align interacting partners in the proximity of each other, and thereby promote their dimerization. For example, LC8 binds to an extended segment of the Swallow protein and promotes its association to a stable dimer with features of a coiled coil (*31*). These experiments were conducted on a 100-amino acid domain, which is monomeric in the absence of LC8 and includes LC8 binding site residues 286−296, and a weakly predicted coiled coil for residues 206−278. Swallow dimerization coupled to LC8 binding is reminiscent of the model for IC dimerization coupled to LC8 binding in Figure 2. If we extend this model to Swallow, the interaction of Swallow with LC8 involves non-dynein-associated LC8 that is essential for Swallow localization because it promotes Swallow conformational stability and dimerization, and presumably orders the structure at the C-terminal domain, which includes the residues necessary for Swallow localization and transport along the cytoskeleton.

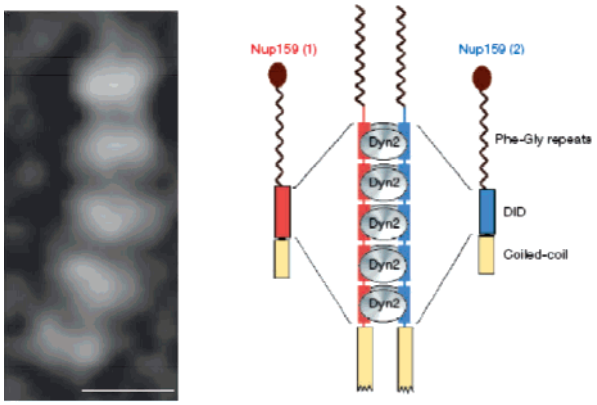

FIGURE 3: Electron microscopy structure of yeast LC8 (termed Dyn2), in complex with DID domain (dynein light chain interacting domain) of Nup159 with a schematic representation of a model of Nup159−Dyn2−Nup159 interaction. Adapted from ref *30* with permission.

Similar observations were made with myosin V and Nup159. Myosin V includes an LC8 binding site predicted to be a disordered segment between two predicted coiled-coil domains. Upon LC8 binding, both flanking coiled coils are stabilized (*32*, *33*). While myosin V is a dimer in the absence of LC8, the authors proposed that LC8 binding brings together the disordered segments from each chain and increases the efficiency of dimerization. In the absence of LC8, myosin V is in a monomer−dimer equilibrium but in the presence of LC8 is either a dimer or in a dimer−tetramer equilibrium (*32*). This LC8-dependent change in the coiled-coil content or dimerization efficiency of myosin V is proposed as the underlying mechanism for the transition between enzymatically active and inactive conformations (*32*). With Nup159, LC8 binds between a Phe-Gly repeat and a predicted coiled-coil domain to form a structure in which five LC8 homodimers align between the two extended chains and stabilize the dimeric coiled coil (*30*), which in turn stabilizes the incorporation of this module into the nuclear pore complex (Figure 3).

Other LC8 binding partners that are dimers or prone to dimerization in the absence of LC8 are nNOS, Trps1, and

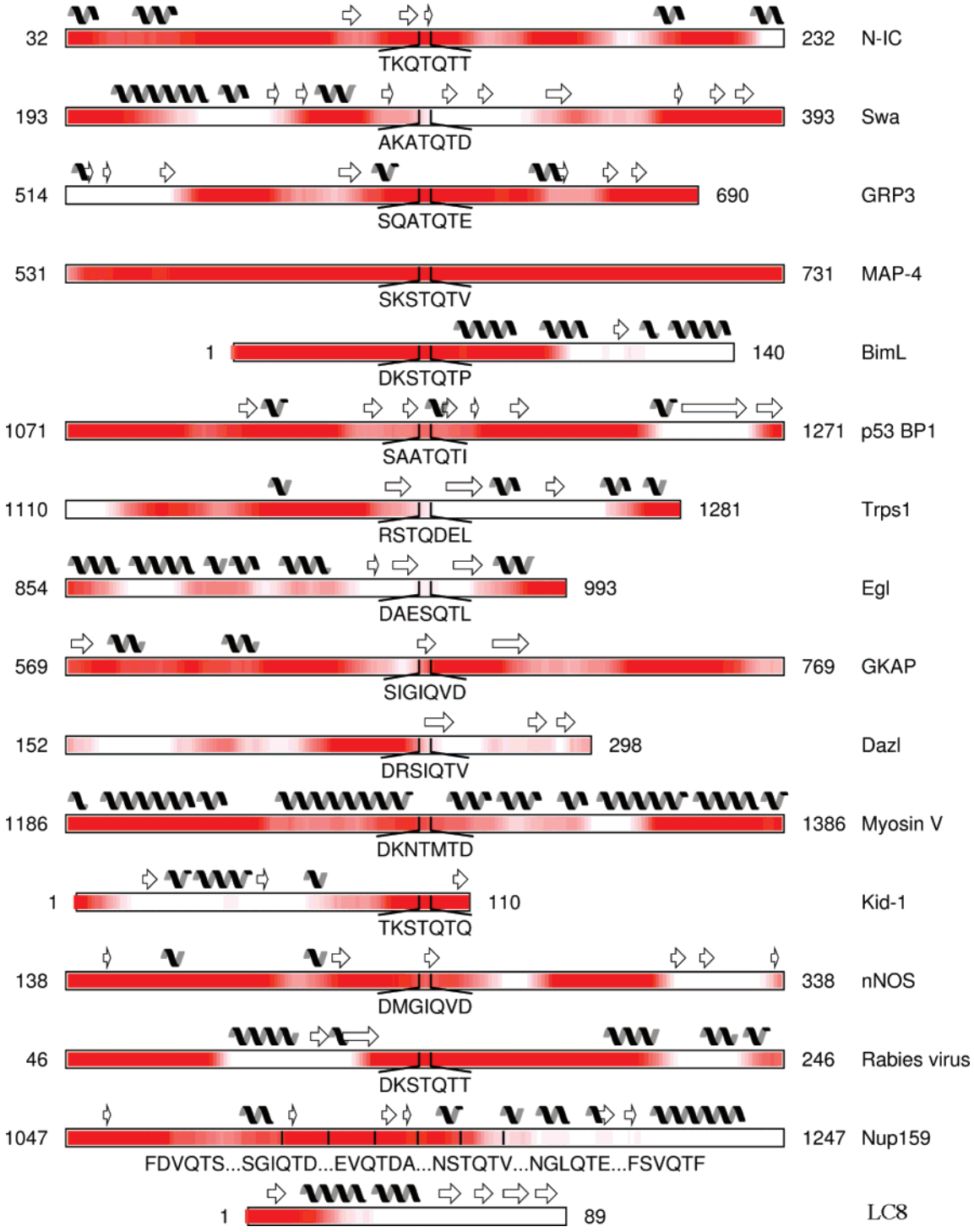

FIGURE 4: Local disorder in the segment corresponding to the LC8 binding site is commonly predicted for LC8 binding partners. The LC8 binding site is shown as a consensus sequence on 15 diverse binding partners. The entire sequence for Kid-1 and BimL is given. For the others, 100 residues flank the N-terminus of the consensus sequence and 100 residues or the rest of the sequence flanks the C-terminus. Predicted secondary structure (helices and sheets) is shown above the diagram for each protein. Predicted disorder is indicated by color, from white (<40% probability of disorder) to red (>60% probability). Binding partners are as follows: N-IC, N-terminal domain of IC; Swa, Swallow protein; GRP3, an exchange factor for Ras-like small GTPases, whose interaction with LC8 is responsible for its subcellular localization (*43*); MAP-4, microtubule-associated protein 4 (*37*); BimL, a proapoptotic Bcl2 family protein (*12*) whose interaction with LC8 promotes apoptosis (*44*); p53 BP1, p53 binding protein 1; Trps1, a repressor of GATA-regulated genes (*13*) whose interaction with LC8 in the cell nucleus results in suppression of its transcriptional repression activity; Egl, Egalitarian; GKAP, guanylate kinase-associated protein neuronal scaffold whose interaction with LC8 is involved in trafficking of the postsynaptic density-95 complex (*45*); Dazl; myosin V; Kid-1, a zinc finger transcription factor expressed in response to renal injury (*37*); nNOS, neuronal nitric oxide synthase for which a crystal structure is available starting from residue 297, consistent with the white observed in the sequence diagram for residues 297−338 and further confirming that residues N-terminal to position 297 are disordered since they are not part of the crystal structure, consistent with prediction; rabies virus phosphoprotein; and Nup159, whose several sequential binding motifs are shown as a vertical line on the sequence diagram. Sequence analysis for LC8 is shown at the bottom for comparison.

Dazl. In nNOS, the segment that contains the LC8 binding site is not part of the dimerization and catalysis domain but is necessary for distinct tissue-specific expression (*34*), suggesting a regulatory role for LC8 in nNOS that is distinct from catalysis. Trps1 has zinc fingers prone to forming homo- and heterodimers (*35*), and preliminary studies on Dazl show that it undergoes both in vivo and in vitro dimerization (*36*). We predict that LC8 would facilitate alignment and increased organization of the disordered segments of these proteins, and/or promote heterodimerization between these proteins and other LC8 binding partners, if both chains contain segments that are prone to protein−protein interactions.

Regulation of LC8 Activity. The essential roles of LC8 in these various complexes raise intriguing questions about how LC8 is recycled in the cell and how it selects different binding partners. Phosphorylation appears to be one likely mechanism in these processes. Phosphorylation of LC8 at Ser 88 (Figure 1) promotes its dissociation to a monomer and subsequent dissociation from its binding partners (*20*), which facilitates LC8 recycling. In addition, phosphorylation allows LC8 to select more tightly bound ligands. Since phosphorylation does not completely dissociate LC8 dimer, tighter binding ligands or higher protein concentrations can shift the monomer−dimer equilibrium and modulate binding activity.

LC8 Binding Partner Sequences Contain Predicted Regions of Local Disorder near the LC8 Binding Site. We have assembled in Figure 4 the sequences of known LC8 binding partners for which there is either clear evidence of binding or an accepted recognition sequence. These LC8 binding partners have diverse roles in the cell, and varying types of subcellular localization, including the cell nucleus, and for many, their activity is regulated by LC8 binding. All the examples discussed above are included in Figure 4. Most binding partners were initially identified by yeast two-hybrid screens and subsequently verified by GST pulldown assays. All binding partners have either a KXTQTX or an XGIQVD binding motif (*37*), or a similar motif with conservative modifications. Sequences for the entire protein are presented for Kid1 and BimL, and sequences flanking the consensus motif are shown for the other proteins. Sequence-based secondary structure and disorder prediction were performed using Jpred (*38*) and RONN (*39*) servers, respectively, as two independent predictors of lack of periodic secondary structure and intrinsic flexibility.

A common theme that emerges from Figure 4 is that the LC8 binding site is within a segment predicted to have either a high degree of local disorder, a lack of periodic secondary structure, or both. For some binding partners such as IC, almost the whole sequence stretch is predicted to be highly disordered, consistent with experimental measurements on a large IC fragment. For others such as Trps1 and myosin V, the predicted disorder is localized to the segments containing the LC8 recognition motif. These proteins are large multidomain, multifunctional proteins, and some such as nNOS contain ordered segments in the rest of the protein not shown in this stretch.

Included at the bottom of Figure 4 is sequence analysis of LC8 for which a crystal structure is determined for the peptide-bound form. The disorder predicted in the N-terminal domain is consistent with structural studies. There is crystallographic disorder in the bound form for residues 1−5 and a high degree of disorder in the first 20 residues by hydrogen exchange criteria in the free protein indicating a high degree of flexibility. Aside from this N-terminal segment, the protein is essentially folded (Figure 1).

What does predicted local disorder imply for the segment within the native protein? A locally disordered segment is a flexible ensemble of interconverting conformations, all or some of which are disordered. Such an ensemble may have all or mostly disordered conformations, or it may have significant fractions of both disordered and ordered conformations.

Hub Proteins and Protein Disorder. Hub proteins can be either completely disordered, partially disordered with disordered loops as well as ordered regions, or ordered but interact with disordered partners (reviewed in ref *22*). Examples of the latter include cyclin-dependent protein kinases, which bind to intrinsically disordered inhibitors p21 and p27 during cell cycle regulation (*40*), and calmodulin, which binds several diverse disordered binding partners (*41*).

We propose that the ability of LC8 to bind diverse proteins via the same binding site is facilitated by local intrinsic disorder and flexibility in the region of the consensus sequence of its binding partners. The versatility of the LC8 binding site is promoted by the fact that the consensus sequence of the bound partner lines up as a sixth β-strand in the LC8 antiparallel β-sheet. Disorder in the unbound partner would favor extended conformations of the consensus sequence, similar to the β-strand conformation of that sequence when it is bound. The binding peptide is likely disordered since the peptide-binding groove is deep and narrow, suggesting it can only accommodate an isolated strand, which by definition could not be prefolded in a β-sheet in the absence of the partner strand. By binding two disordered partner molecules and aligning them in the proximity of each other in an orientation conducive to mutual interactions, LC8 facilitates partner structural organization and dimerization. In this view, LC8 is an example of an essentially ordered hub protein for which binding to locally disordered partner proteins is linked to their ordering and dimerization.

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REFERENCES

1. Vallee, R. B., Williams, J. C., Varma, D., and Barnhart, L. E. (2004) Dynein: An ancient motor protein involved in multiple modes of transport, *J. Neurobiol. 58*, 189−200.
2. Mische, S., Li, M., Serr, M., and Hays, T. S. (2007) Direct observation of regulated ribonucleoprotein transport across the nurse cell/oocyte boundary, *Mol. Biol. Cell 18*, 2254−2263.
3. Harris, T. J., and Peifer, M. (2005) The positioning and segregation of apical cues during epithelial polarity establishment in *Drosophila*, *J. Cell Biol. 170*, 813−823.
4. Stokin, G. B., and Goldstein, L. S. (2006) Axonal transport and Alzheimer's disease, *Annu. Rev. Biochem. 75*, 607−627.
5. Dick, T., Ray, K., Salz, H. K., and Chia, W. (1996) Cytoplasmic dynein (ddlc1) mutations cause morphogenetic defects and apoptotic cell death in *Drosophila melanogaster*, *Mol. Cell. Biol. 16*, 1966−1977.

6. Makokha, M., Hare, M., Li, M. G., Hays, T., and Barbar, E. (2002) Interactions of cytoplasmic dynein light chains Tctex-1 and LC8 with the intermediate chain IC74, *Biochemistry 41*, 4302−4311.
7. Lo, K. W. H., Naisbitt, S., Fan, J. S., Sheng, M., and Zhang, M. J. (2001) The 8-kDa dynein light chain binds to its targets via a conserved (K/R)XTQT motif, *J. Biol. Chem. 276*, 14059−14066.
8. Lo, K. W., Kan, H. M., Chan, L. N., Xu, W. G., Wang, K. P., Wu, Z., Sheng, M., and Zhang, M. (2005) The 8-kDa dynein light chain binds to p53-binding protein 1 and mediates DNA damage-induced p53 nuclear accumulation, *J. Biol. Chem. 280*, 8172−8179.
9. Espindola, F. S., Suter, D. M., Partata, L. B. E., Cao, T., Wolenski, J. S., Cheney, R. E., King, S. M., and Mooseker, M. S. (2000) The light chain composition of brain Myosin-Va: Calmodulin, myosin-II essential light chains, and 8-kDa dynein light chain/PIN, *Cell Motil. Cytoskeleton 47*, 269−281.
10. Jaffrey, S. R., and Snyder, S. H. (1996) PIN: An associated protein inhibitor of neuronal nitric oxide synthase, *Science 274*, 774.
11. Schnorrer, F., Bohmann, K., and Nusslein-Volhard, C. (2000) The molecular motor dynein is involved in targeting Swallow and bicoid RNA to the anterior pole of *Drosophila* oocytes, *Nat. Cell Biol. 2*, 185−190.
12. Puthalakath, H., Huang, D. C. S., O'Reilly, L. A., King, S. M., and Strasser, A. (1999) The proapoptotic activity of the Bcl-2 family member Bim is regulated by interaction with the dynein motor complex, *Mol. Cell 3*, 287−296.
13. Kaiser, F. J., Tavassoli, K., Van den Bemd, G. J., Chang, G. T. G., Horsthemke, B., Moroy, T., and Ludecke, H. J. (2003) Nuclear interaction of the dynein light chain LC8a with the TRPS1 transcription factor suppresses the transcriptional repression activity of TRPS1, *Hum. Mol. Genet. 12*, 1349−1358.
14. Raux, H., Flamand, A., and Blondel, D. (2000) Interaction of the rabies virus P protein with the LC8 dynein light chain, *J. Virol. 74*, 10212−10216.
15. Fan, J. S., Zhang, Q., Tochio, H., Li, M., and Zhang, M. J. (2001) Structural basis of diverse sequence-dependent target recognition by the 8 kDa dynein light chain, *J. Mol. Biol. 306*, 97−108.
16. King, S. M., Barbarese, E., Dillman, J. F., PatelKing, R. S., Carson, J. H., and Pfister, K. K. (1996) Brain cytoplasmic and flagellar outer arm dyneins share a highly conserved M_r 8,000 light chain, *J. Biol. Chem. 271*, 19358−19366.
17. Wu, H., Maciejewski, M. W., Takebe, S., and King, S. M. (2005) Solution structure of the Tctex1 dimer reveals a mechanism for dynein-cargo interactions, *Structure 13*, 213−223.
18. Liang, J., Jaffrey, S. R., Guo, W., Snyder, S. H., and Clardy, J. (1999) Structure of the PIN/LC8 dimer with a bound peptide, *Nat. Struct. Biol. 6*, 735−740.
19. Benison, G., Karplus, P. A., and Barbar, E. (2007) Structure and dynamics of LC8 complexes with KXTQT-motif peptides: Swallow and dynein intermediate chain compete for a common site, *J. Mol. Biol. 371*, 457−468.
20. Song, Y., Benison, G., Nyarko, A., Hays, T. S., and Barbar, E. (2007) Potential role for phosphorylation in differential regulation of the assembly of dynein light chains, *J. Biol. Chem. 282*, 17272−17279.
21. Han, J. D., Bertin, N., Hao, T., Goldberg, D. S., Berriz, G. F., Zhang, L. V., Dupuy, D., Walhout, A. J., Cusick, M. E., Roth, F. P., and Vidal, M. (2004) Evidence for dynamically organized modularity in the yeast protein-protein interaction network, *Nature 430*, 88−93.
22. Dunker, A. K., Cortese, M. S., Romero, P., Iakoucheva, L. M., and Uversky, V. N. (2005) Flexible nets. The roles of intrinsic disorder in protein interaction networks, *FEBS J. 272*, 5129−5148.
23. Haynes, C., Oldfield, C. J., Ji, F., Klitgord, N., Cusick, M. E., Radivojac, P., Uversky, V. N., Vidal, M., and Iakoucheva, L. M. (2006) Intrinsic disorder is a common feature of hub proteins from four eukaryotic interactomes, *PLoS Comput. Biol. 2*, e100.
24. Nyarko, A., Hare, M., Hays, T. S., and Barbar, E. (2004) The intermediate chain of cytoplasmic dynein is partially disordered and gains structure upon binding to light-chain LC8, *Biochemistry 43*, 15595−15603.
25. Benison, G., Nyarko, A., and Barbar, E. (2006) Heteronuclear NMR Identifies a Nascent Helix in Intrinsically Disordered Dynein Intermediate Chain: Implications for Folding and Dimerization, *J. Mol. Biol. 362*, 1082−1093.
26. Lo, K. W. H., Kan, H. M., and Pfister, K. K. (2006) Identification of a novel region of the cytoplasmic dynein intermediate chain important for dimerization in the absence of the light chains, *J. Biol. Chem. 281*, 9552−9559.
27. Navarro, C., Puthalakath, H., Adams, J. M., Strasser, A., and Lehmann, R. (2004) Egalitarian binds dynein light chain to establish oocyte polarity and maintain oocyte fate, *Nat. Cell Biol. 6*, 427−435.
28. Lee, K. H., Lee, S., Kim, B., Chang, S., Kim, S. W., Paick, J. S., and Rhee, K. (2006) Dazl can bind to dynein motor complex and may play a role in transport of specific mRNAs, *EMBO J. 25*, 4263−4270.
29. Tan, G. S., Preuss, M. A., Williams, J. C., and Schnell, M. J. (2007) The dynein light chain 8 binding motif of rabies virus phosphoprotein promotes efficient viral transcription, *Proc. Natl. Acad. Sci. U.S.A. 104*, 7229−7234.
30. Stelter, P., Kunze, R., Flemming, D., Hopfner, D., Diepholz, M., Philippsen, P., Bottcher, B., and Hurt, E. (2007) Molecular basis for the functional interaction of dynein light chain with the nuclear-pore complex, *Nat. Cell Biol. 9*, 788−796.
31. Wang, L., Hare, M., Hays, T. S., and Barbar, E. (2004) Dynein light chain LC8 promotes assembly of the coiled-coil domain of swallow protein, *Biochemistry 43*, 4611−4620.
32. Wagner, W., Fodor, E., Ginsburg, A., and Hammer, J. A., III (2006) The binding of DYNLL2 to myosin Va requires alternatively spliced exon B and stabilizes a portion of the myosin's coiled-coil domain, *Biochemistry 45*, 11564−11577.
33. Hodi, Z., Nemeth, A. L., Radnai, L., Hetenyi, C., Schlett, K., Bodor, A., Perczel, A., and Nyitray, L. (2006) Alternatively spliced exon B of myosin Va is essential for binding the tail-associated light chain shared by dynein, *Biochemistry 45*, 12582−12595.
34. Panda, K., Adak, S., Aulak, K. S., Santolini, J., McDonald, J. F., and Stuehr, D. J. (2003) Distinct influence of N-terminal elements on neuronal nitric-oxide synthase structure and catalysis, *J. Biol. Chem. 278*, 37122−37131.
35. McCarty, A. S., Kleiger, G., Eisenberg, D., and Smale, S. T. (2003) Selective dimerization of a C2H2 zinc finger subfamily, *Mol. Cell 11*, 459−470.
36. Ruggiu, M., and Cooke, H. J. (2000) In vivo and in vitro analysis of homodimerisation activity of the mouse Dazl1 protein, *Gene 252*, 119−126.
37. Rodriguez-Crespo, I., Yelamos, B., Roncal, F., Albar, J. P., Ortiz de Montellano, P. R., and Gavilanes, F. (2001) Identification of novel cellular proteins that bind to the LC8 dynein light chain using a pepscan technique, *FEBS Lett. 503*, 135−141.
38. Cuff, J. A., Clamp, M. E., Siddiqui, A. S., Finlay, M., and Barton, G. J. (1998) JPred: A consensus secondary structure prediction server, *Bioinformatics 14*, 892−893.
39. Yang, Z. R., Thomson, R., McNeil, P., and Esnouf, R. M. (2005) RONN: The bio-basis function neural network technique applied to the detection of natively disordered regions in proteins, *Bioinformatics 21*, 3369−3376.
40. Lacy, E. R., Wang, Y., Post, J., Nourse, A., Webb, W., Mapelli, M., Musacchio, A., Siuzdak, G., and Kriwacki, R. W. (2005) Molecular basis for the specificity of p27 toward cyclin-dependent kinases that regulate cell division, *J. Mol. Biol. 349*, 764−773.
41. Marlow, M. S., and Wand, A. J. (2006) Conformational dynamics of calmodulin in complex with the calmodulin-dependent kinase kinase α calmodulin-binding domain, *Biochemistry 45*, 8732−8741.
42. DeLano, W. L. (2002) *The PyMOL Molecular Graphics System*, DeLano Scientific, San Carlos, CA.
43. Okamura, S. M., Oki-Idouchi, C. E., and Lorenzo, P. S. (2006) The exchange factor and diacylglycerol receptor RasGRP3 interacts with dynein light chain 1 through its C-terminal domain, *J. Biol. Chem. 281*, 36132−36139.
44. Vadlamudi, R. K., Bagheri-Yarmand, R., Yang, Z., Balasenthil, S., Nguyen, D., Sahin, A. A., den Hollander, P., and Kumar, R. (2004) Dynein light chain 1, a p21-activated kinase 1-interacting substrate, promotes cancerous phenotypes, *Cancer Cell 5*, 575−585.
45. Naisbitt, S., Valtschanoff, J., Allison, D. W., Sala, C., Kim, E., Craig, A. M., Weinberg, R. J., and Sheng, M. (2000) Interaction of the postsynaptic density-95/guanylate kinase domain-associated protein complex with a light chain of myosin- V and dynein, *J. Neurosci. 20*, 4524−4534.

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