Multivalency in the Assembly of Intrinsically Disordered Dynein Intermediate Chain

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Cytoplasmic dynein is a 1.2-MDa microtubule-associated motor protein complex involved in Golgi maintenance, nuclear migration, mitotic spindle formation, and organelle positioning and transport (1). Dynein has multiple subunits in the 10–500-kDa molecular mass range (2): two heavy chains, two light intermediate chains, two intermediate chains (IC),2 and three light chains known in Drosophila melanogaster as Tctex1, LC8, and LC7. The three light chains are homodimers when active, and each binds at a separate site on the N-terminal domain of IC (see Fig. 1a).

Molecular genetic analyses in D. melanogaster indicate that IC serves an essential function (3), consistent with its multiple roles in dynein assembly, regulation, and binding to cargo. Its C-terminal domain (C-IC) provides the sites for assembly of heavy chains, whereas its N-terminal domain (N-IC) provides binding sites for diverse light chains, for the p150Glued subunit of dynactin (4, 5), and for several other proteins presumed to be cellular cargo such as herpes simplex virus protein (6), the CIC-2 chloride channel (7) and β-catenin (8). In vivo disruption of dynnein-dynactin interaction affects Golgi complex and endosome organization (9, 10). In vitro studies of various segments of N-IC indicate that it is natively disordered and monomeric (11, 12); two N-IC chains bind to both Tctex1 and LC8, and within each chain the 10–12-amino acid amino acid recognition sequence undergoes a disorder-to-order transition (13) to form α and β strands incorporated into a β-sheet at the light chain dimer interface (see Fig. 1 and supplemental Fig. S1) (14, 15).

Tctex1 and LC8 are homologs (see supplemental Fig. S1) that bind at adjacent highly conserved IC recognition sequences (see supplemental Table S1). In addition to binding IC, both associate with a wide variety of proteins once presumed to be dynein cargo linked by Tctex1 or LC8 to the dynein motor. However, recent structural data (14, 15) challenge this view because the LC8 dimer (and by analogy the Tctex1 dimer (15)) binds either two chains of IC or two chains of putative cargo proteins at the same location (14, 16), making the adaptor hypothesis much less attractive. An alternative hypothesis is that LC8 is a hub protein essential for promoting the dimerization and physiological activity of its diverse protein partners, including IC (17).

Such a regulatory role for these light chains in dynein assembly has been suggested based on the evidence that LC8 binding induces IC/IC self-association (13), and that the binding affinity of LC8 to IC is enhanced when Tctex1 is present (15). In vivo studies (18) of various truncations of N-IC show that an IC lacking the light chains recognition sequence binds less efficiently to p150Glued and is less effective at inhibiting dynein-based transport than a longer IC containing the light chains recognition sequence and the second predicted coiled-coil. In such experiments, overexpressed IC is presumed to bind free dynactin and competes with endogenous dynein IC resulting in perturbed microtubule organization and centrosome integrity. A similar experiment using deletions of N-IC shows the intact C-IC is not sufficient for efficient binding to the heavy chain, but requires both light chain binding sites and the second coiled-coil (9). The importance of both light chains for dynein function is clear; unknown are the mechanisms by which the two light chains work together to enhance the binding of IC to p150Glued on one end and to the dynein heavy chain on the other.
Multivalency in Disordered Dynein Intermediate Chain

To determine the potential role of Tctex1 and LC8 in IC assembly and regulation, we measure here the thermodynamics of formation of the IC-Tctex1-LC8 system, along with the model system IC-LC8-LC8. Mutual enhancement is expected as prior binding of IC to either light chain produces a bivalent IC duplex. The thermodynamics of these complexes and the structural organization of IC promote the formation of an IC duplex that serves as a polybivalent scaffold for dynein assembly and illustrate a novel aspect of the LC8-driven dimerization of a new class of intrinsically disordered proteins.

EXPERIMENTAL PROCEDURES

Protein Preparation—A synthetic peptide corresponding to IC residues 123–138 was purified as described earlier (19). D. melanogaster ICTL, Tctex1 and LC8 were prepared as described earlier (11, 13, 20, 21). The IC_LL gene was produced by Gene-Script (Piscataway, NJ). Purity was verified by SDS-PAGE and matrix-assisted laser desorption ionization time-of-flight mass spectrometry: IC_LL molecular mass = 7893.1 Da (7897.5 theoretical). Sequence-based calculation of absorptivity at 280 nm was used to measure protein concentrations (22).

Isothermal Titration Calorimetry—Proteins were dialyzed in buffer containing 50 mM sodium phosphate, 50 mM sodium chloride, 1 mM sodium azide, pH 7.5. Thermodynamics of binding were determined at 20, 25, 30, and 35 °C using a VP-ITC calorimeter (MicroCal, Northampton, MA). Data were processed using the manufacturer’s supplied software package, Origin 7.0 (OriginLab Corp., Northampton, MA), and fit to a single-binding site model, A + B → AB, where A refers to a single IC chain and B refers to a single protomer of Tctex1 or LC8. Heat of dilution, estimated to be equal to the enthalpy of single IC chain and B refers to a single protomer of Tctex1 or LC8. Heat of dilution, estimated to be equal to the enthalpy of single IC chain and B refers to a single protomer of Tctex1 or LC8. Mutual enhancement is expected as a function of temperature. All ΔH values were obtained with linear correlation coefficients of >0.98. Calculated changes in heat capacity at constant pressure (ΔCp) were determined from the change in enthalpy (ΔH) as a function of temperature. All ΔCp values were obtained with linear correlation coefficients of >0.98. Calculated changes in heat capacity at constant pressure (ΔCp) were computed as described by Spolar and Record (24). Changes in solvent accessible surface area were determined using SURFACE RACER 5.0 (25). Protein data bank structures 2P2T (14), 3FM7 (Tctex1 site only), 3FM7 (LC8 site only), and 3GLW were used to model apo-ICTL-LC8, pre-bound ICTL:; Tctex1, pre-bound ICTL-LC8, and ICTL-LC8 complexes, respectively.

Size Exclusion Chromatography and Multiangle Light Scattering—Association states were determined from analytical size exclusion chromatography (Superdex 75) with an online multiangle light scattering detector (miniDawn, Wyatt Technology) in 200 mM sodium sulfate, 50 mM sodium phosphate, pH 7.3. Data were processed using ASTRA version 5.1.9.1 (Wyatt Technology). The molecular mass of apo-ICTL and the ICTL/Tctex1/LC8 complex were 7.7 and 68.7 kDa, respectively, in good agreement with the theoretical molecular masses for a monomer (7.8 kDa) and a dimer of heterotrimer (67.3 kDa), respectively.

X-ray Crystallography—An IC construct (corresponding to residues 92 to 260) bound to Tctex1 and LC8 (WT IC-Tctex1-LC8) and ICTL-LC8-LC8 complex were in buffer containing 15 mM sodium chloride, 5 mM Tris, pH 7.5, and a final protein complex concentration of 0.2 mM. Crystals were obtained at 4 °C using hanging drops setup with a 1-μl protein and reservoir solution equilibrated against a 400-μl reservoir.

For WT IC-Tctex1-LC8, hexagonal pyramidal crystals were obtained using a reservoir of 16% PEG 8000, 100 mM sodium cacodylate, 200 mM calcium acetate, pH 6.5. Crystals grew to a final size of 0.16 × 0.16 × 0.3 mm³. For ICTL-LC8-LC8, blade-shaped crystals were obtained using 30% PEG 400, 100 mM Hepes, 200 mM magnesium chloride, pH 7.5. Crystals grew to a final size of 0.15 × 0.15 × 0.5 mm³ (Supplemental Table S2). Crystals for both complexes were analyzed by SDS gel after data collection and showed that the full-length IC constructs were proteolyzed. Therefore both complexes contain an IC domain of unknown length N- and C-terminal to the Tctex1 and LC8 binding sites. IC is natively disordered and sensitive to protease degradation, it seems likely that formation of the crystal lattice occurred opportunistically during in situ proteolysis (26), and future attempts at reproducing these crystals may be aided by the addition of protease to the protein solution.

Crystals were pulled through oil before flash-freezing in loops using liquid nitrogen. For WT IC-Tctex1-LC8, 3.5 Å resolution oscillation data (Δφ = 1.0°) were collected at the Berkeley Advanced Light Source, HHMI beam line 8.2.2. For ICTL-LC8-LC8, 3.15 Å resolution oscillation data were collected using an in-house Raxis IV system with CuKα radiation. Data were integrated using Mosflm (27) and scaled using SCALA (28). The WT IC-Tctex1-LC8 and ICTL-LC8-LC8 space groups were P62, with unit cell of a = b = 115.66 Å, c = 90.37 Å and P62, with a = b = 44.65 Å, c = 219.38 Å, respectively. Phases were determined for both crystals by molecular replacement using PHASER (29) and 2PG1 and 3BRI as search models.

The WT IC-Tctex1-LC8 crystal had a full IC-Tctex1-LC8 complex (six protein chains) in the asymmetric unit. Molecular replacement placed a dimer of Tctex1 and a dimer of LC8 allowing an electron density map to be calculated that showed density for the IC peptides. IC residues were manually added using Coot (30) and the complex was refined using REFMAC (31). The ICTL-LC8-LC8 crystal had just one-fourth of the ICTL-LC8-LC8 complex (one LC8 chain and one-half of an ICTL chain) in the asymmetric unit. Molecular replacement placed a protomer of LC8 in the cell and the resulting electron density map revealed density for the portion of IC interacting with LC8 and weaker density for the five-residue IC linker connecting LC8 protomers in adjacent unit cells. The five IC linker residues, modeled in an extended PPII conformation, fit well in the distance between the LC8 dimers and improved Rfree. The
linker IC residues were modeled at half-occupancy because only half of the unit cells should contain these residues.

For both structures one domain per chain was used for TLS refinement. Crystallographic data collection and refinement statistics are summarized in supplemental Table S2.

Accession Codes—The atomic coordinates and structure factors for WT IC-Tctex1-LC8 and ICLL-LC8 have been deposited into the PDB with accession numbers 3FM7 and 3GLW.

RESULTS

Design of IC Constructs—Three IC constructs were used (Fig. 1b): ICTL, a wild-type sequence corresponding to IC residues 84 –143 with both Tctex1 and LC8 recognition sequences (T and L refer to sites for binding Tctex1 and LC8, respectively); ICTL, a mutated version of the same segment with the Tctex1 recognition sequence replaced by the LC8 recognition sequence; and IC5, a shortened sequence corresponding to IC residues 123–138 with only one LC8 recognition sequence. The ICTL, ICLL, and ICTL constructs do not include the IC regions predicted to be coiled-coils (13). Tctex1 and LC8 bind the longer IC constructs that contain either predicted coiled-coil with similar affinity as ICTL (data not shown) and therefore for simplicity and for ease of comparison of ITC and x-ray crystallography data, these studies focus on ICTL. The apo-IC constructs used are all monomeric and disordered, whereas both Tctex1 and LC8 are dimeric and bind two monomers of IC. When fully assembled, the complexes formed by the IC constructs are, respectively, ICTL-Tctex1-LC8, ICLL-LC8, and ICTL-LC8, and LC8.

Thermodynamics of ICTL-Tctex1-LC8 Complex Formation—Representative isothermal titration calorimetry (ITC) data are shown in Fig. 2. Association parameters (ΔG°, ΔH°, -TΔS°) and the related heat capacity change (ΔCpexp) are given in Table 1 and supplemental Fig. S2. For ICTL, we measured the binding of Tctex1 to both apo-ICTL and the pre-bound ICTL-LC8 complex and similarly the binding of LC8 to apo-ICTL and to pre-bound ICTL-Tctex1. Control experiments showed that in the absence of IC there is no interaction between Tctex1 and LC8 (data not shown).

Apo-ICTL binds Tctex1 or LC8 with a similar affinity (Kd of 8 μM), and a ΔCpexp of −0.42 and −0.26 kcal/mol/K, respectively (see Table 1 and supplemental Fig. S2, b and c). When ICTL is pre-bound to either Tctex1 or LC8, the second light chain binds with higher affinity (Kd of 0.2 μM), and results in a more negative ΔCpexp of −0.59 and −0.41 kcal/mol/K, for Tctex1 and LC8, respectively (see Table 1 and supplemental Fig. S2, d and e). Between the first and second binding events, there is a 50-fold (ΔΔG° of −2.4 kcal/mol) binding affinity enhancement accompanied by a change in ΔCpexp (ΔΔCpexp of −0.16 kcal/mol/K). The more negative ΔCpexp suggests additional burial of surface area that accompanies the second binding event (24).

Interestingly, LC8 binds IC5 and apo-ICTL with indistinguishable affinities and ΔCpexp values (see Table 1), indicating that...
binding is limited to the short recognition sequence of IC and that there is no change in rigidity solvent accessible surface area in regions of IC not in direct contact with LC8.

**Thermodynamics of ICLL-LC8-LC8 Complex Formation**—To examine the origin of the binding enhancement associated with pre-bound ICLL relative to apo-ICLL, we engineered an IC construct, ICLL, which has the Tctex1 binding site replaced with a fragment of IC bound to Tctex1 and LC8 solved at 3.5 Å resolution shows homodimers of Tctex1 and LC8 binding structure of a fragment of IC bound to Tctex1 and LC8 solved at 3.5 Å resolution shows homodimers of Tctex1 and LC8 binding.

**Crystal Structures of the Ternary Complexes**—The crystal structure of a fragment of IC bound to Tctex1 and LC8 solved at 3.5 Å resolution shows homodimers of Tctex1 and LC8 binding to IC.

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**TABLE 1**

Thermodynamic parameters for association of IC constructs with dimeric LC8 and Tctex1 at 25 °C

| Interaction | Protein | Ligand | $K_d$ | $\Delta G_0$ | $\Delta H_0$ | $-T\Delta S_0$ | $\Delta C_p_{exp}$ | $\Delta C_p_{calc}^a$ |
|-------------|---------|--------|-------|-------------|-------------|----------------|----------------|----------------|
| LC8         | ICLL    | 8.0 ± 2.1 | -7.0 ± 0.1 | 1.1 ± 0.3 | -8.1 ± 0.4 | -0.26 | -0.27 |
| Tctex1      | ICLL    | 9.6 ± 3.6 | -6.8 ± 0.2 | -6.9 ± 0.8 | 0.1 ± 0.5 | -0.42 | -0.56 |
| LC8         | LC8     | 8.0 ± 1.5 | -7.0 ± 0.1 | -13.7 ± 0.6 | 6.7 ± 0.2 | -0.26 | -0.27 |
| Tctex1      | LC8+ICL | 0.21 ± 0.09 | -9.1 ± 0.2 | -8.8 ± 0.3 | -0.3 ± 0.7 | -0.59 | -0.56 |
| LC8         | ICLL    | 0.13 ± 0.09 | -9.4 ± 0.3 | -14.5 ± 0.3 | 5.2 ± 0.4 | -0.41 | -0.25 |
| Tctex1      | ICLL    | 0.50 ± 0.06 | -9.0 ± 0.1 | -13.7 ± 0.2 | 4.8 ± 0.2 | -0.26 | -0.25 |

$^a$ $\Delta C_p_{calc}$ values were computed as described by Spolar and Record (24) based on the surfaces buried by a light chain monomer binding to a single IC chain.

$^b$ No structure is reported for IC with Tctex1 in the absence of LC8, so $\Delta C_p_{calc}$ for apo-ICLL-Tctex1 was determined from the WT IC-Tctex1-LC8 complex (PDB 3FM7).

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**FIGURE 3.** Crystal structure of the ternary complexes of IC constructs with light chains. Semi-transparent surface and secondary structural elements are shown for (a) the WT IC-Tctex1-LC8 structure reported here (PDB entry 3FM7), (b) the ICLL-LC8/LC8 structure previously reported (PDB entry 2PG1) (15), and (c) the ICLL-LC8-LC8 structure reported here (PDB 3GLW). In 2PG1, Tctex1 and LC8 form a small contact surface due to a bend in IC not observed in the structures reported here, implying that the IC linker remains flexible in the bound complex. The ICLL-LC8-LC8 crystal had one LC8 chain and one half of an ICLL chain in the asymmetric unit, as discussed under “Experimental Procedures”, the (d) center of the asymmetric unit and interpreted model of the ICLL-LC8-LC8 structure are shown outlined in *blue*. For all figures Tctex1 is yellow, LC8 is green, and IC chains are *black* and *white*. Data collection and refinement statistics are given in supplemental Table S2. The figure was generated using PyMOL (45).

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**33118 JOURNAL OF BIOLOGICAL CHEMISTRY**

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linker region, there is no density for residues N- or C-terminal to the LC8 recognition sequences. As with the WT IC-Tctex1-LC8 complex, the IC_{1L} linker adopts a P_{II} conformation and has no IC/IC or light chain/light chain contacts.

**DISCUSSION**

Tctex1 and LC8 Have Mutually Enhanced Binding Affinities for IC—Three constructs, IC_{1L}, having binding sites for two LC8 subunits, IC_{T1}, having binding sites for Tctex1 and LC8, and IC_{L}, having a single LC8 binding site, provide a powerful system for quantitatively dissecting bivalency effects in this multicomponent assembly complex. The full thermodynamic cycle for IC_{T1} ternary complex formation is shown in Fig. 4 along with the corresponding binding steps in IC_{1L} and IC_{L}. The second light chain binds IC_{T1,LL}LC8 or IC_{T1,Tctex1} with affinity enhancement of about 50-fold (\(\Delta G^0\) of −2.4 kcal/mol). This is consistent with and extends a hydrogen exchange study that indicated enhanced binding of at least 10-fold for the second light chain, i.e. \(\Delta G^0 \leq -1.4\) kcal/mol (15). Interestingly, LC8 binds IC_{1L,LL}LC8 with affinity enhancement of about 1000-fold (\(\Delta G^0\) of −4.0 kcal/mol).

A Pure Chelate Effect in IC_{1L,LL}LC8 Binding—The 1000-fold affinity enhancement of the second LC8 binding to IC_{1L,LL} agrees well with a 3-order of magnitude enhancement estimated from calculations of the entropic barrier to binding in bivalent small molecules (32). It is due entirely to a favorable change in the association entropy with a minimal enthalpic change between the first and second binding events (\(\Delta H^0 = 0\)) (Fig. 5a). This enhancement is an entropic multivalency effect known in a number of biological systems as the chelate effect (33–36). The first LC8 dimer connects two equivalent IC_{1L} chains resulting in a bivalent IC_{1L} duplex that has significantly higher binding affinity for the second LC8 dimer than for the first (supplemental Fig. S3). The second binding event is of higher affinity because the unfavorable loss of translational and rotational entropy is fully paid in the first binding step, so that the subsequent binding step does not incur this entropic penalty. The enhancement in the IC_{1L,LL} system is a remarkable demonstration of the solely entropic origin of the chelate effect in a multisubunit protein assembly. It suggests that the alignment of the two IC arms is optimal for the second LC8 binding.

Deviation from a Purely Entropic Chelate Effect in Wild-type—The 50-fold affinity enhancement of LC8 with IC_{1L,Tctex1} is less than the full chelate enhancement observed for LC8 with IC_{1L,LL}LC8 and suggests that additional unfavorable interactions occur during the second binding event that offset the full gain from multivalency.

As pointed out by Jencks (37), underestimation of the free energy contribution from the chelate effect is due to destabilizing interactions in a bivalent system that do not occur in the equivalent monovalent systems, because contributions from the chelate effect and the destabilizing interactions are additive. The 2.4 kcal/mol enhancement of binding seen for the IC_{TL} system implies that the full entropic enhancement of 4.0 kcal/mol realized in the IC_{1L,LL} model system is reduced by 1.6 kcal/mol.

The lower enhancement is accompanied by a \(\Delta \Delta C_{P exp}\) of −0.16 kcal/mol/K associated with the second binding (see Figs. 4 and 5, b and c). Because a change in heat capacity is commonly associated with burial of the nonpolar surface, the implication of the \(\Delta \Delta C_P\) value is that the surface area buried in the ternary...
Multivalency in Disordered Dynein Intermediate Chain

complex is larger than in the binary complex with either Tctex1 or LC8.

Structural Basis for the Destabilizing Interactions—To identify the site that undergoes additional structural changes, we compared ΔCp,calc for each step in the thermodynamic cycle to ΔCp,exp derived from empirical calculations based on surface areas buried in the complexes (24). Crystal structures for IC1-LC8, IC1Tctex1-LC8, and IC1L-LC8-LC8 have a similar IC-LC8 interface each yielding a ΔCp,calc near 0.26 kcal/mol/K. For Tctex1, the crystal structure of IC1Tctex1-LC8 yields a ΔCp,calc of −0.56 kcal/mol/K for interactions in the ternary complex, but no crystal structure is available to guide the calculation for a binary complex of IC1Tctex1.

As seen in Fig. 4, ΔCp,exp and ΔCp,calc agree very well for apo-IC1Tctex1 binding LC8, and for IC1Tctex1-LC8 binding Tctex1. They are also in good agreement for IC1 with LC8 and apo-IC1L with LC8. The only discrepancies occur on the left side of the IC1L thermodynamic cycle. For apo-IC1Tctex1 binding Tctex1, ΔCp,exp is less negative than the ΔCp,calc of 0.56 kcal/mol/K (based on the ternary complex), and for the second step of IC1Tctex1-LC8 binding LC8, ΔCp,exp is more negative than ΔCp,calc by a similar difference of 0.16 kcal/mol/K. A simple model explaining these data is that less of IC1T becomes buried when it is in a binary complex with Tctex1 (explaining the less negative ΔCp,exp of the first step), and this portion becomes buried during LC8 binding (explaining the more negative ΔCp,exp of the second step).

Analysis of the crystal structure suggests that the part of IC not interacting with Tctex1 in the binary complex is the C-terminal end of the recognition site. When LC8 binds IC1-Tctex1, the two C-terminal ends of the Tctex1 recognition site would be pulled in closer to the Tctex1 surface, but the N-terminal ends should not be affected. The ΔCp,calc values match ΔCp,exp if the last four residues of the Tctex1 recognition site (NIPP in D. melanogaster) remain solvent exposed in the binary IC1Tctex1-Tctex1 complex and then become bound during LC8 binding. Interestingly these last four residues of the Tctex1 recognition site have high sequence conservation (supplemental Table S1). We therefore attribute the lower enhancement of LC8 binding to IC1Tctex1 versus IC1L-LC8 to additional disorder-to-order transition at the IC1T-Tctex1 interface, with the last four residues of the Tctex1 recognition site acting as an attenuator of the favorable bivalency effects of the system.

Disorder and Flexibility in IC Complexes—When natively disordered apo-IC (13) binds to dimeric LC8 and Tctex1, IC recognition sequences form two extended β-strands at the light chain interfaces (see Fig. 3) (14, 15). The linker connecting the interfaces is elongated, but ordered enough to show appreciable electron density in the crystal structure. In WT IC1-Tctex1-LC8 and IC1L-LC8-LC8 structures, the IC linker assumes a coil conformation, commonly observed in unfolded peptides (38). In a previously reported structure of Rattus norvegicus IC peptide bound to D. melanogaster Tctex1 and LC8 (15), Tctex1 and LC8 contact each other due to a bend in the IC linker (see Fig. 3b). Williams et al. (15) noted the contact surface is small and occurs through non-conserved Tctex1 and LC8 residues. In our D. melanogaster WT IC1-Tctex1-LC8 and IC1L-LC8-LC8 structures no contact is observed (see Fig. 3, a and c). Together these results indicate that the IC in the linker region of the ternary complex is flexible, and can therefore sample the different orientations observed in both crystal structures of Fig. 3, a and b.

Evolution of Adjacent Sites—Tctex1 and LC8 are homologs that have similar IC binding sites in vertebrates IC (SKVTQV and SKETQT in Danio rerio). Both observations raise the prospect that there may have been an ancestral IC that bound either two LC8 dimers or two Tctex1 dimers. Interestingly, adjacent LC8 sites are common in real systems. In guanylate kinase-associated protein (39) and the nuclear pore protein (Nup159) (40), for example, there are two and six adjacent LC8 recognition sequences, respectively. Because Tctex1 and LC8 bind apo-IC with similar affinities, the evolutionary selection of two distinct light chains is unlikely to be due just to enhanced affinity. Indeed, an enhancement lower than the full multivalent effect suggests affinity has been modulated in the dynein IC assembly to optimize the balance of stability and reversibility. Consistent with this, contiguous sites for both Tctex1 and LC8 along with the linker separating them are observed in euukaryotes (supplemental Table S1).

Polybivalency in Dynein Assembly and Regulation—The emerging picture of dynein IC is one of an elongated, flexible scaffold that contains a number of binding sites for attachment of bivalent dynein light chains (see Fig. 1), the dimeric p150Glued subunit of dynactin (18), and various cargo proteins. Bivalency arises because alignment of two IC chains results in a duplex with multiple additional bivalent sites. We refer to this as a polybivalent scaffold, which will be created when apo-IC binds any of its bivalent ligands, or forms a self-associated coiled-coil (13, 15). Multiple bivalent sites provide the potential for mutual enhancement of affinity for every additional ligand bound, as well as for coiled-coil interchain interactions (see Fig. 1). For any two sites, the extent of binding enhancement depends on the length of the linker between them. A short linker such that connecting Tctex1 and LC8 will result in higher local effective concentrations and higher binding enhancement than a longer linker such as that connecting LC8 and the weakly predicted coiled-coil.

A polybivalent system can be quite stable even when the association constant of any single ligand is moderate to weak. Although the assemblage is stable, multivalency, combined with flexibility, provides reversible binding of ligands, a property very useful for regulation and functional adaptability (41). The resultant dynein N-IC assembly, endowed by multivalency, retains disorder and associated flexibility; together these provide versatility and reversibility in response to changes in local cellular environment.

A polybivalent assembled IC can explain the considerably higher binding efficiency of p150Glued to a fragment of IC containing four bivalent sites (the N-terminal predicted coiled-coil, the light chains binding site, and the central predicted coiled-coil) relative to a fragment that contains only one bivalent site (the N-terminal coiled-coil domain) (18). Any of the three additional sites enhances coiled-coil interchain interactions resulting in a tighter binding to p150Glued. A similar explanation applies to the more efficient binding to the heavy chain of an IC fragment containing the light chains binding site in addition to the WD repeat C-terminal domain (9).
These insights into dynein IC assembly are relevant to other non-dynein proteins that bind LC8. Analysis of these non-dynein proteins led to the hypothesis that LC8 acts as a hub protein that promotes dimerization of its binding partners (17). One such protein, Nup 159, when bound to LC8, is expected to be an aligned and elongated dimer on which one or more additional bivalent binding sites are available (17), we propose that, like IC, many of the LC8 binding partners become polybivalent scaffolds and that the IC/light chain system is a canonical example of polybivalence in the assembly of a new class of intrinsically disordered proteins.

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