Role of Disorder in the Regulation of Dynein Intermediate Chain

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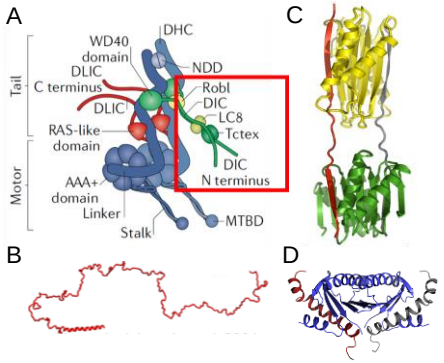
Abstract

Cytoplasmic dynein is a large, eukaryotic motor protein complex composed of heavy, intermediate, light intermediate, and light chain subunits. The motor domain of dynein has been well characterized, however in the tail domain there are almost 300 amino acids of disorder that remain missing from resolved dynein structures. This disorder lies in dynein's intermediate chain (IC), the key protein in dynein's cargo attachment subcomplex, involved in binding with dynein's light chain subunits as well as various non-dynein regulatory proteins. Two of dynein's regulatory proteins, dynactin's p150^{Glued} subunit and a nuclear distribution protein (NudE) have both been shown to bind to the N-terminus, single α-helix of IC in a variety of species. In this work, we characterize the binding interaction between IC and coiled-coiled segments of p150^{Glued} and NudE in *Chaetomium thermophilum* (CT), a thermophilic filamentous fungus and a novel choice for dynein studies. With the use of isothermal titration calorimetry (ITC), analytical ultracentrifugation (AUC), and nuclear magnetic resonance (NMR) we have explored the importance of the level of disorder in a transient, secondary helix (H2) in IC's binding affinity to regulatory proteins. Furthermore, by studying both a small, 88 residue construct of IC, as well as a much larger 260 residue construct, we have demonstrated that multivalency and disorder underlie the regulation of the IC/p150^{Glued} and IC/NudE interactions.

Background

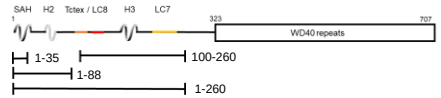
Dimeric dynein light chains bring together two disordered, monomeric chains of N-IC to create a subcomplex scaffold



(A) Much of dynein is structurally well characterized[1]. The cargo attachment subcomplex however, (boxed in red) has much more ambiguity due to its disorder and flexibility. (B) The N-term of IC is an almost completely disordered monomeric chain. (C-D) Solved crystal structures of Tctex and LC8 (C) and LC7 (D) in complex with IC. The dimeric light chains bring together two chains of IC to form a polybivalent scaffold. IC adopts light chain structure at the binding sites, while linkers remain disordered [2-10].

N-IC is disordered

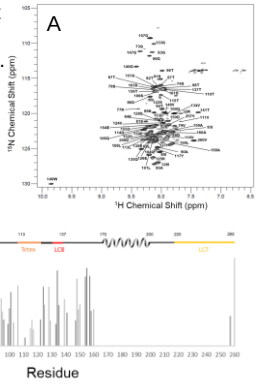
Dynein intermediate chain domain architecture



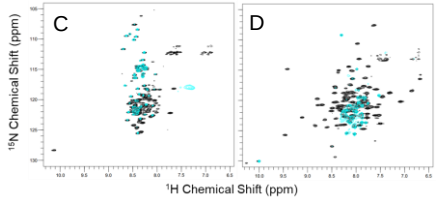
IC from *Chaetomium thermophilum* (CT IC) is predicted to have an N-terminal single α-helix (SAH), a transient/nascent second helix (H2), and a more C-terminal helix (H3). Based on sequence motifs, the Tctex (orange), LC8 (red), and LC7 (yellow) binding sites are predicted to be in regions similar to Drosophila IC. The C-terminal region is predicted to contain seven WD40 repeat domains. IC_{1-88}, IC_{1-260}, $IC_{100-260}$, and $IC_{140-260}$ show the constructs used in this work. IC_{1-35} is a construct used in prior work.

Characterization of disorder in CT IC_{1-260} by NMR

(A) ^{15}N-^{1}H TROSY-HSQC spectra of IC_{1-260} acquired at 800 MHz at 10˚C with assignments labeled. (B) Relative intensities for assigned residues plotted beneath the domain architecture for IC_{1-260}.

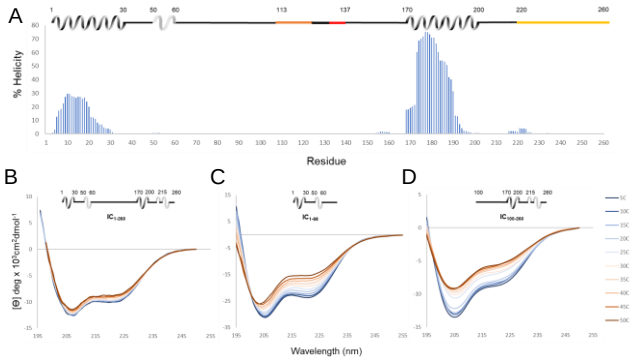


(C-D) Overlays of a CLEANEX experiment (light blue) onto the ^{15}N-^{1}H TROSY-HSQC spectrum (black) for both 10˚C (C) and 40˚C (D).



IC_{1-260} is stabilized by a H3

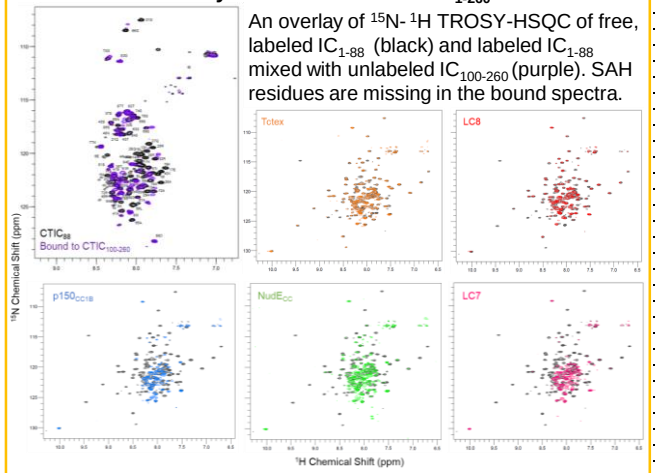
Secondary structure and stability of CT IC Constructs



(A) Agadir helix prediction of IC_{1-260} , plotting %Helicity by residue. Temperature-dependent CD spectra of (B) IC_{1-260}, (C) IC_{1-88}, and (D) $IC_{100-260}$. Spectral shape for all constructs indicates a mixture of α-helical secondary structure as well as regions of intrinsic disorder. Loss in structure, or lack thereof, over a temperature range of 5-50˚C indicate how each construct varies in stability.

Long-range interactions between IC_{1-260} Helixes

Tertiary Contacts within CT IC_{1-260}



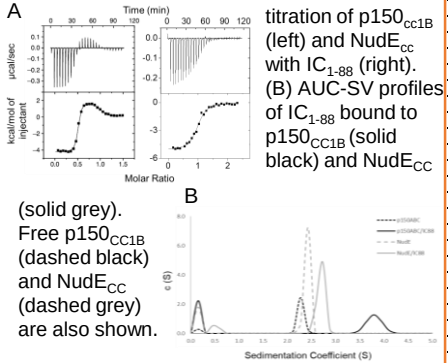
An overlay of ^{15}N-^{1}H TROSY-HSQC of free, labeled IC_{1-88} (black) and labeled IC_{1-88} mixed with unlabeled $IC_{100-260}$ (purple). SAH residues are missing in the bound spectra.

Overlays of free IC_{1-260} (black) and binding partners at 40˚C. Binding patterns of partners bound at the N-term (p150 & NudE) match that of LC7 bound at the C-term. Binding at sights more central linker regions (Tctex & LC8) show their own distinct pattern.

IC/p150 & IC/NudE Interactions

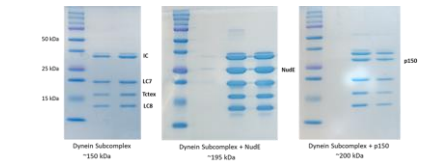
Binding interactions of CT IC_{1-88} with $p150_{CC1B}$ and $NudE_{CC}$

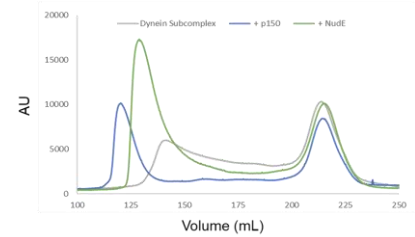
(A) Representative ITC thermograms (top) and binding isotherms (bottom) from the titration of $p150_{cc1B}$ (left) and $NudE_{cc}$ with IC_{1-88} (right). (B) AUC-SV profiles of IC_{1-88} bound to $p150_{CC1B}$ (solid black) and $NudE_{CC}$ (solid grey). Free $p150_{CC1B}$ (dashed black) and $NudE_{CC}$ (dashed grey) are also shown.



Subcomplex Reconstitution

Purification and characterization of reconstituted dynein subcomplexes

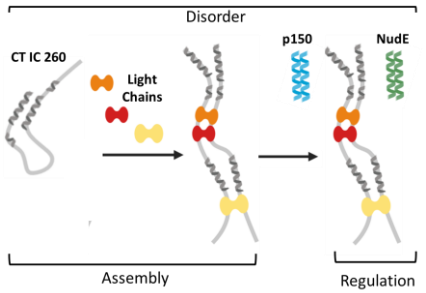




SEC traces of the dynein subcomplex (IC/light chains) (grey) and the dynein subcomplex with $p150_{CC1B}$(blue) or $NudE_{CC}$ (green). SDS-PAGE gels of fractions collected from SEC for all complexes showing all expected proteins

Conclusions

Disorder, assembly, and regulation in the dynein attachment subcomplex



From our studies with CT IC_{1-260} we conclude:

- CT IC_{1-260} is largely disordered with regions of helicity that impart long-range tertiary contacts between the N and C-terminus
- Tertiary contacts cause free CT IC_{1-260} to adopt a more compact structure that is stabilizing compared to smaller constructs
- This construct of IC allows for study of complex binding interactions, including the successful reconstitution of various forms of fully bound subcomplexes.
- Binding of the three dynein light chains cause CT IC_{1-260} to form an extended polybivalent scaffold and bind to $p150_{CC1B}$ and $NudE_{CC}$ with greater affinity than free CT IC_{1-260} alone.
- Disorder and assembly of the cargo attachment subcomplex are critical to its regulation

Acknowledgements

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