A conserved interaction of the dynein light intermediate chain with dynein-dynactin effectors necessary for processivity

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Cytoplasmic dynein is the major minus-end-directed microtubule-based motor in cells. Dynein processivity and cargo selectivity depend on cargo-specific effectors that, while generally unrelated, share the ability to interact with dynein and dynactin to form processive dynein–dynactin-effector complexes. How this is achieved is poorly understood. Here, we identify a conserved region of the dynein Light Intermediate Chain 1 (LIC1) that mediates interactions with unrelated dynein–dynactin effectors. Quantitative binding studies map these interactions to a conserved helix within LIC1 and to N-terminal fragments of Hook1, Hook3, BICD2, and Spindly. A structure of the LIC1 helix bound to the N-terminal Hook domain reveals a conformational change that creates a hydrophobic cleft for binding of the LIC1 helix. The LIC1 helix competitively inhibits processive dynein-dynactin-effector motility in vitro, whereas structure-inspired mutations in this helix impair lysosomal positioning in cells. The results reveal a conserved mechanism of effector interaction with dynein–dynactin necessary for processive motility.
Cytoplasmic dynein 1 (dynein) is the major minus-end-directed microtubule-based motor in eukaryotic cells. It is responsible for the transport of very diverse cargoes from the periphery to the center of the cell, including lysosomes, mitochondria and autophagosomes. Recent work has shown that both cargo-specificity and processivity depend on the interaction of dynein with its general adaptor, the dynactin complex, and a series of cargo-specific effectors, including BICD2, Hook1, Spindly, FIP3 and NIN/NINL. These proteins are generally unrelated at the sequence level, but they all contain large portions of predicted coiled-coil structure and share the ability to interact with both dynein and dynactin to activate processive motility. It remains unclear, however, whether each effector has evolved these functions independently or whether they share common structural-functional features and similar interactions with dynein and dynactin. Here, we show that a conserved amphipathic helix within the unstructured C-terminal region of the dynein Light Intermediate Chain 1 (LIC1) interacts with diverse dynein–dynactin effectors. The interactions were quantitatively characterized using purified proteins and isothermal titration calorimetry (ITC). A crystal structure of the LIC1 helix in complex with the N-terminal Hook domain of Hook3 reveals a conformational change within the Hook domain that gives rise to a hydrophobic cleft where the LIC1 helix binds. Supporting the importance of the LIC1-effector interaction, we found that the LIC1 helix competitively inhibits the processive motility of dynein–dynactin in complex with either Hook3 or BICD2 in single-molecule assays using total internal reflection fluorescence (TIRF) microscopy. Finally, in cellular assays, mutating the LIC1 helix leads to defective dynein-driven positioning of lysosomes. Together, the results reveal the existence of a conserved mechanism of interaction between functionally unrelated dynein–dynactin effectors and the dynein LIC1, which is required for processive dynein-driven transport.

Results
Hook interacts with the dynein LIC1 via the Hook domain. The dynein LICs, comprising two closely related isoforms (LIC1 and LIC2), consist of two domains—an N-terminal GTPase-like domain that interacts with the dynein heavy chain and a less conserved and predicted unstructured C-terminal region, referred to here as the effector-binding domain. Using pull-down studies, it had been previously shown that the LIC1-effector-binding domain interacts with several dynein–dynactin effectors, including Hook3, FIP3, BICD2, and Spindly. On the other hand, a group of dynein-binding proteins, including BICD2, Spindly, HAP1, and TRAK share a coiled-coil segment, termed the CC1-Box, that has been directly implicated in LIC1 binding. Here, we set out to specifically map and quantitatively characterize the interactions of LIC1 with several dynein–dynactin effectors, including Hook1, Hook3, BICD2, and Spindly. Hook1 and Hook3 are known dynein effectors that function in endosomal transport. We expressed truncated constructs of human Hook1 and Hook3 in E. coli, whereas full-length Hook1 was expressed in insect cells (Fig. 1a, b). Because Hook contains several regions of predicted coiled-coil (CC1–4) (Fig. 1a), we first analyzed whether these constructs were dimeric or monomeric using light scattering. A construct corresponding to the N-terminal Hook domain (Hook111–166) was monomeric, whereas construct Hook111–443, extending to the end of CC2 was dimeric (Fig. 1c). In contrast, Hook111–239, comprising only the globular Hook domain and CC1 region, was in equilibrium between dimers and monomers, as indicated by an experimentally measured mass of 39.7 kDa, i.e., intermediate between those of the dimer and the monomer. Full-length human LIC1 was expressed as a fusion protein with MBP to increase its solubility, and was also found to be monomeric by light scattering (Fig. 1c).

Using ITC, MBP-LIC1FL bound Hook111–443 with low micromolar affinity ($K_D = 8.1 \mu M$) and ~1:1 stoichiometry, i.e., two LIC1 molecules per Hook1 dimer (Fig. 1d). Note that this ITC titration was performed at 30 °C, instead of 20 °C for most titrations performed here, because the amount of heat given off by this reaction was too small to allow for reliable fitting of the thermodynamic parameters. Consistent with the light scattering results, the titration of Hook111–239 into buffer produced a significant endothermic reaction, which was interpreted as indicative of dimer dissociation, with a $K_D$ of 2.1 $\mu M$ (Fig. 1e).

This conclusion was confirmed by analysis of Hook111–239 on a gel. A dimeric construct stabilized through the addition of a GCN4 leucine zipper at the C terminus, whose titration into buffer did not produce any significant heat change (Fig. 1e). Hook111–239 bound MBP-LIC1FL with a $K_D$ of 12.9 $\mu M$ and ~1:1 stoichiometry (Fig. 1f), which is very similar to what was observed with Hook111–443 (Fig. 1d) despite the fact that the titration was inverted by placing Hook111–239 in the syringe and MBP-LIC1FL in the cell. The monomeric construct Hook111–166 also bound MBP-LIC1FL with similar affinity ($K_D = 12.7 \mu M$) and 1:1 stoichiometry. Together these results show that: (a) the LIC1-binding site is fully contained within the conserved N-terminal Hook domain, (b) each Hook dimer interacts with two LIC1s, and (c) the CC1 region of Hook forms an unstable coiled-coil, which on its own cannot support stable Hook dimerization.

To gain further insights into the overall structure of Hook and the disposition of the Hook domain with respect to the coiled-coil segments, we used rotary shadowing electron microscopy to visualize full-length Hook1 (Fig. 1h). Hook had a kinesin-like appearance, with most particles displaying two well-separated globular domains at one end, connected through a short neck-like region to a long thin rod, which was often interrupted by a pronounced kink, followed by a shorter thin rod. These features were interpreted to correspond to the N-terminal Hook domain, the unstable CC1 region, CC2, the central so-called Spindly motif, and CC3, respectively (Fig. 1i). The smaller C-terminal cargo-binding domain (CBD) was only occasionally visualized as a defined structural feature (Fig. 1i). This assignment of domains is consistent with the length of the segment extending from the end of the neck region to the central kink, whose mean length of

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**Fig. 1** Hook interacts with LIC1 via the N-terminal Hook domain. **a** Domain organization of Hook1 and constructs used in this study (CC: coiled-coil, CBD: cargo-binding domain). **b** SDS-PAGE (4–12%) showing several of the proteins used in this study. **c** SEC-MALS analysis of Hook1 constructs and MBP-LIC1FL (color coded as indicated). The molar mass determined from light scattering (right y-axis) and the UV absorption at 280 nm (left y-axis) are plotted as a function of the elution volume. The theoretical masses are given in parenthesis. **d**–**g** ITC titrations of LIC1 and Hook1 constructs as indicated. Listed with each titration are the concentrations of the protein in the syringe and in the cell, as well as the temperature of the experiment and parameters of the fit (stoichiometry, $N$, dissociation constant $K_D$). Errors correspond to the s.d. of the fits. Open symbols correspond to titrations into buffer (except panel e, where both titrations are into buffer). **h** Representative rotary shadowing EM image of Hook111–443. Scale bar, 100 nm. **i** Close-up view of a representative Hook111–443 molecule shown alongside a cartoon representation of the Hook1 domains based on the rotary shadowing EM, secondary structure and sequence conservation analyses (see Supplementary Fig. 1). Scale bar, 50 nm. **j** Length distribution of the region spanning from the end of the neck to the kink. Bin size, 5 nm, $n = 33$.
~31 nm approximately corresponds to the predicted dimensions of CC2 (~27 nm) (Fig. 1j), as estimated from the structures of other coiled-coil proteins. The dimensions of the remaining smaller domains cannot be accurately measured at this resolution. The assignment of domains is also consistent with structural predictions and sequence conservation analyses, showing a series of coiled-coil segments (CC1, CC2, CC3, and CC4) interspersed with three globular regions (Hook domain, Spindly motif, and CBD), connected by short, unstructured loops of lower sequence conservation (Supplementary Fig. 1). The variability of the kink angle between CC2 and CC3 suggests that the regions N- and C-terminal to the Spindly motif move relatively independently of
each other, i.e., the Spindly motif appears to function as a ‘hinge’. Finally, the fact that the two globular Hook domains appear well separated from each other in most of the particles visualized is consistent with the two helices that form the CC1 segment (neck) not forming a stable coiled-coil, as also suggested by the light scattering (Fig. 1c) and ITC (Fig. 1e) results.

A helix in LIC1 C-terminal region binds the Hook domain. The C-terminal effector-binding domain of LIC1 (human LIC1 residues 390–523) has been shown to interact with dynein–dynactin effectors, including Hook3, BICD2, and Spindly9,11,12. However, it was unknown whether different effectors bound to the same or different regions on the LIC1 C terminus, and these interactions...
were characterized by qualitative rather than quantitative analyses. Here, we set out to map the specific region of the LIC1 C terminus implicated in interactions with Hook and other effectors (see below) and quantitatively characterize the interactions. Most of the LIC1-effector-binding domain is predicted to be unstructured and, unlike the GTPase-like domain, it is not highly conserved among species (Supplementary Fig. 2). However, sequence analysis reveals two regions of relatively high conservation that coincide with predicted α-helical segments, which we named Helix-1 (human LIC1 residues 440–456) and Helix-2 (residues 493–502) (Fig. 2a and Supplementary Fig. 2). To test whether these conserved helical segments participate in the interaction with the Hook domain, we generated two C-terminally truncated LIC1 constructs, MBP-LIC1(1–461), which removes the region C-terminal to Helix-1 and MBP-LIC1(1–437), which additionally removes Helix-1 (Fig. 2a). MBP-LIC1(1–437) failed to bind the Hook domain by ITC (Fig. 2c), whereas MBP-LIC1(1–461) bound the Hook domain (Fig. 2b) with nearly the same affinity (K_D = 10.1 μM) as MBP-LIC1FL (K_D = 12.7 μM) (Fig. 1g). These results suggested that the binding site is contained within Helix-1. Consistent with this conclusion, the Hook domain failed to bind to construct MBP-LIC1F447A,F448A, in which two strictly conserved phenylalanine residues in the middle of Helix-1 were simultaneously mutated to alanine (Fig. 2d).

To further test the role of Helix-1 in Hook binding, we expressed a 26-a.a. peptide (LIC1(433–458)), extending several amino acids N- and C-terminally to the predicted helical segment to ensure proper folding of Helix-1. The Hook domain of Hook1 bound to LIC1(433–458) with nearly the same affinity (K_D = 15.7 μM) as to MBP-LIC1FL (Figs. 1g and 2c). The Hook domain of Hook3 (human Hook3 residues 1–160) also bound MBP-LIC1FL and Helix-1 with similar affinities (Fig. 2e). These affinities were comparable to those observed with the Hook domain of Hook1 (Figs. 1g and 2c). Together, these results map the LIC1–Hook interaction to the conserved Helix-1 within the effector-binding domain of LIC1 and the N-terminal Hook domain of both Hook1 and Hook3. Furthermore, the conserved hydrophobic residues F447 and F448 within Helix-1 likely form part of the binding interface.

**Structure of a complex of the Hook domain and the LIC1 helix.**

To further understand the mechanism of interaction between Hook and LIC1, we determined the crystal structure of human Hook31–160 in complex with human LIC1 Helix-1 at 1.5 Å resolution (Fig. 3a–c and Table 1). The electron density is well defined for Hook3 residues 10–160 and LIC1 residues 441–454 (Fig. 3b). The first nine amino acids of Hook3 and residues 433–440 and 455–458 of Helix-1 were disordered and are, thus, unlikely to participate in the interaction. As previously reported, the Hook domain displays a canonical 7-helix calponin homology (CH)-like fold, featuring an additional helix at the C terminus termed helix ø8. Generally, the structure superimposes well with that of the unbound Hook domain determined previously, with an r.m.s. deviation of 1.4 Å for 136 equivalent Ca atoms (Fig. 3d). However, the Hook domain-specific helix ø8, which in the unbound structure is fully extended and interacts in anti-parallel fashion with the same helix from a symmetry-related molecule in the crystal, is broken into two helices (ø8α and ø8β) in the current structure (Fig. 3d), giving rise to a V-shaped hydrophobic cleft that constitutes the binding site for LIC1 Helix-1 (Fig. 3c). As predicted, the visualized portion of Helix-1 is folded as an amphipathic α-helix, with its hydrophobic surface facing the hydrophobic cleft of the Hook domain (Fig. 3c). All the highly conserved, hydrophobic amino acids of the LIC1-effector-binding domain are directly inserted into the hydrophobic cleft of the Hook domain, including L444, F447, F448, and L451, explaining why the mutant MBP-LIC1F447A,F448A failed to bind the Hook domain (Fig. 2d). The binding interface also coincides with the most highly conserved surface of the Hook domain (Fig. 3e).

The Hook domain also interacts with a second LIC1 Helix-1 from a neighboring complex in the crystal lattice (Fig. 3e). This interaction presents the less conserved, hydrophilic surface of Helix-1 to a less conserved surface on the Hook domain, which a priori is inconsistent with a native interaction. Yet, to rule out this interaction, we generated two Hook domain mutants: A138D, testing the presumed crystal packing contact, and M140D, testing the anticipated native binding site (Fig. 3f). The Hook31–160A138D mutant bound MBP-LIC1FL with the same affinity as wild type Hook31–160 (compare Figs. 2f and 3g), whereas the Hook31–160M140D mutant failed to bind MBP-LIC1FL (Fig. 3h), confirming that the native binding site of Helix-1 is located at the interface between ø8α and ø8β, and conferring functional significance to the conformational change that splits helix ø8 into two helices. Indeed, even in the presence of Helix-1, we obtained a second crystal form showing the reported extended conformation of helix ø89, but the LIC1 peptide was not bound in these crystals. To further test the importance of the conformational change in helix ø8 for LIC1 binding, we generated a truncated construct, Hook31–143, lacking the ø8β portion of helix ø8, i.e., the region that bends back to form the V-shaped cleft (Fig. 3d). Hook31–143 failed to bind MBP-LIC1FL (Fig. 3i). Collectively, these results confirm that the extended helix ø8 of the Hook domain, which distinguishes this domain from the canonical CH fold, undergoes a conformational change to produce a conserved, hydrophobic cleft for binding of the conserved LIC1 Helix-1.

**The LIC1 helix binds diverse dynein–dynactin effectors.** Next, we asked whether LIC1 Helix-1 was also implicated in interactions with other dynein–dynactin effectors that are generally structurally and functionally unrelated to each other. As mentioned above, a recent study found that a group of dynein–dynactin effectors share a region termed the CC1-Box that was implicated in LIC1 binding through pull-down and mutagenesis studies12. To test whether LIC1 Helix-1 also mediates the interaction with CC1-Box-containing effectors, we expressed N-terminal fragments of two effectors: BICD21–98 and Spindly1–142 (Fig. 4a, b). These constructs extend N- and C-terminally from the CC1-Box to include the first predicted coiled-coil segment of each

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**Fig. 2** The conserved Helix-1 within the LIC1-effector-binding domain binds the Hook domain. a Alignment of LIC sequences from different species and isoforms around the predicted Helix-1 within the C-terminal effector-binding domain (top) and domain diagram of human LIC1 showing the constructs used in this study (bottom). The name of each sequence includes the organism of origin and UniProt accession code. Yellow and orange backgrounds indicate 70% and 100% sequence conservation, respectively. Red stars highlight residues F447 and F448 that were mutated to alanine. The predicted Helix-1 and Helix-2, coinciding with regions of higher sequence conservation (see Supplementary Fig. 2), are highlighted in the domain diagram, and Helix-1 is also depicted above the sequence alignment. The region corresponding to the Helix-1 (LIC1(433–458)) peptide is contoured red. b c ITC titrations of Hook31–160 and Hook31–160 into LIC1 constructs (as indicated). Listed with each titration are the concentrations of the protein in the syringe and in the cell, as well as the temperature of the experiment and parameters of the fit (stoichiometry N, dissociation constant K_D). Errors correspond to the s.d. of the fits. Open symbols correspond to control titrations into buffer.
Fig. 3 Crystal structure of the Hook domain in complex with LIC1 Helix-1. a Ribbon and surface representation of the structure of Hook31–160 (magenta) in complex with Helix-1 (LIC1433–458, blue). The side chains of Helix-1 are shown using a sticks representation, colored by atom type. b Close-up view of the Helix-1 binding site, showing the 2Fo-Fc electron density map (blue mesh) at 1.5 Å resolution, contoured at 1σ around an all-atom representation of Helix-1. c Close-up view of the Helix-1 binding site, showing the residues at the hydrophobic contact interface. d Superimposition of the structure of the Hook domain from the Helix-1-bound complex (magenta) and unbound structure (gray). A conformational change in the C-terminal helix α8, which distinguishes this domain from the CH domain, leads to the formation of two helices (α8a and α8b) that constitute the binding site for Helix-1. e Sequence conservation of the Hook domain (see also Supplementary Fig. 1) mapped onto the surface of the structure and colored from low to high conservation using a red to green gradient. In the crystal lattice, the Hook domain contacts a second Helix-1 from a neighboring complex (light blue). f Surface representation of the Hook domain (magenta), showing in yellow the two amino acids mutated (A138D and M140D) to test the functional relevance of the two Helix-1 interactions. g–i ITC titrations of the indicated Hook31–160 mutants into MBP-LIC1FL. Experimental conditions and fitting parameters are listed. Errors correspond to the s.d. of the fits. Open symbols correspond to titrations into buffer at 20°C.
protein and ensure proper dimerization (note that when bound to dynein–dynactin, the entire N-terminal region of these two proteins appears to form uninterrupted coiled-coil structures16, as depicted in Fig. 4a). Indeed, as verified by light scattering (Fig. 4c), both BICD21–98 and Spindly1–142 form stable coiled-coil dimers, with experimentally determined masses approximately double those calculated from sequence. By ITC, the titrations of LIC1133–454 into BICD21–98 (Fig. 4d) and Spindly1–142 (Fig. 4e) fitted best to two-binding-site isotherms. The affinities of the two binding sites were similar to each other, and they were also similar for the two effectors (with $K_D$s ranging from 1.5 to 7.6 μM). Curiously, however, despite sharing a similar CCI-Box and displaying similar affinities for LIC1 Helix-1, the titrations into BICD21–98 and Spindly1–142 had different overall appearances (Fig. 4d, e). For the Spindly1–142 titration, in particular, the two binding sites have very close affinities and are probably saturated at the same time, but the first site has a mild endothermic character, whereas the second site has a strong exothermic character, which masks the endothermic signal of the first part of the titration, explaining the peculiar shape of this reaction. Likely, LIC1 binding produces different types of conformational changes in these two proteins, which other than the CCI-Box share no apparent sequence similarity. These results confirm that LIC1 Helix-1 constitutes a common binding site for unrelated dynein–dynactin effectors, including CCI-Box-containing effectors (BICD, Spindly) and Hook-family effectors.

### The LIC1 helix/effecter interaction is crucial for motility

To test the functional significance of the LIC1 Helix-1 interaction with dynein–dynactin effectors, we utilized an in vitro single-molecule approach to track the movement of dynein–dynactin-effector complexes obtained from cell extracts using TIRF microscopy8. Lysates of HeLa cells expressing Halo-tagged Hook31–552 labeled with TMR-HaloTag ligand were flowed into a chamber containing Taxol-stabilized microtubules immobilized on coverslips. The dynein-driven motility of single molecules was then monitored both in the absence or the presence of increasing concentrations of Helix-1 or Helix-1-F447A,F448A, a peptide carrying the two mutations found to inhibit binding of full-length LIC1 to the Hook domain (Fig. 2d). Consistent with previous reports5,9, in the absence of Helix-1 we observed robust motility of Halo-Hook31–552-positive complexes along microtubules, characterized by long run lengths and high velocities (Fig. 5a). In contrast, we observed a marked inhibition of processive motility with the addition of Helix-1, with nearly complete inhibition at Helix-1 concentrations of 100 μM or higher, whereas the addition of Helix-1-F447A,F448A did not inhibit motility (Fig. 5a). Similar results were observed in experiments that tracked the movement of dynein–dynactin-BICD2 complexes obtained from cell extracts expressing Halo-BICD21–572 labeled with TMR-HaloTag ligand (Fig. 5b). In this case, however, higher concentrations of Helix-1 (>200 μM) were required for full inhibition, which is not entirely unexpected for in trans competition of an intramolecular interaction.

To assess whether the LIC1-effector interaction contributes to organelle motility in cells, we analyzed the distribution of lysosomes in HeLa cells expressing GFP, LIC1WT-GFP, or the mutant LIC1F447A,F448A-GFP that does not interact with Hook1 (Fig. 2d). Importantly, this mutation is predicted to also block the interaction of LIC1 with other effectors, since we found that Helix-1 is involved in interactions with several effectors (Figs. 2e, g and 4d, e). Lysosomes are well-characterized cargoes of dynein, which drives perinuclear clustering of lysosomes near microtubule minus ends17,18, and LIC1 is known to be required for this activity19. Compared to the expression of GFP alone, the expression of LIC1WT-GFP did not significantly change the distribution of lysosomes, visualized by anti-LAMPI staining of cells fixed 18–22 h after transfection. In contrast, the expression of the LIC1F447A,F448A-GFP mutant resulted in an abnormal localization of lysosomes (Fig. 5c). In these cells, lysosomes appeared dispersed throughout the cytoplasm and did not show the characteristic perinuclear clustering seen in control cells (Fig. 5c). In a blind analysis, LIC1F447A,F448A-GFP-expressing cells displayed a significantly higher percentage of abnormally positioned lysosomes compared to cells expressing GFP or LIC1WT-GFP (Fig. 5d). Together, these results show that the LIC1-effector interaction mediated by Helix-1, and specifically residues F447 and F448, is absolutely required for processive dynein-based motility in vitro and in cells.

### Discussion

Cytoplasmic dynein is responsible for most cellular activities requiring microtubule minus-end-directed motility. However, in isolation, dynein is not processive5,20. It is now recognized that dynein’s functional diversity, including cargo-specificity and processivity, depends on its interaction with the general adaptor dynactin, regulated by an ever-expanding family of dynein–dynactin effector proteins, including BICD22–23, Hook1/35,69, Spindly5, FIP33, and NIN/NINL10. These proteins have been distinctly called adaptors5,9 or regulators21. We have used here the more general term ‘effectors’ because they do both—they help bring together dynein and dynactin and recruit specific cargoes, which are typical adaptor functions, but they also activate dynein processivity, thus playing a regulatory role. We have a limited understanding of how dynein–dynactin effectors exert these
diverse functions, and the lack of recognizable sequence similarity or a common dynein–dynactin-binding motif among all of them has limited our ability to establish general structural-functional correlations. Most of the effectors, however, appear to have cargo-specific binding domains toward their C-termini\textsuperscript{12,14,22}.

Another structural feature shared by all the known effectors is the presence of long regions of coiled-coil. Cryo-EM\textsuperscript{16,26} has limited our ability to establish general structural-functional correlations. Most of the effectors, however, appear to have cargo-

Evidence for the third type of interaction, involving dynein's LIC1 subunit and N-terminal sequences in varous effectors, has so far been limited to pull-down\textsuperscript{9,11,12} and proteomics\textsuperscript{10} studies. Here, we have mapped this interaction to a conserved helix (Helix-1) within the otherwise unstructured and poorly conserved C-terminal region of LIC1. We have further shown that Helix-1 mediates the interaction with structurally and functionally unrelated effectors, and that the interactions typically have low

![Diagram](image-url)
micromolar affinities. We were also able to visualize the structural basis of this interaction at high-resolution for the Hook subfamily of effectors (comprising three isoforms, Hook1–3). Finally, we demonstrated that this interaction enhances the processive motility of dynein in vitro and that disruption of the LIC1-effector interface affects organelle transport in cells. Somewhat analogous to our findings, an interaction between the light and intermediate chains of yeast dynein has been implicated in dimerization and processive motility34.

Curiously, the LIC1-effector interaction involves a conserved motif on the LIC1 side of the interface, but different surfaces on the effector side. For Hook-family effectors, the interaction involves the N-terminal Hook domain, which has a globular fold related to the CH domain. However, it is the extended helix α8 of
theHook domain (absent in the CH fold) that mediates the interaction by forming a V-shaped hydrophobic cleft after splitting into two helices. In BICD2 and Spindly, the interaction involves the CC1-Box, which forms part of a longer coiled-coil segment (Fig. 4a). Conceivably, the two helices of the coiled-coil could separate, partially exposing the hydrophobic core of the coiled-coil to create symmetric binding sites for LIC1 Helix-1 on both sides of the coiled-coil. This would give rise to a binding site that is different in sequence, but possibly structurally similar to that of the Hook domain.

The effectors analyzed here bind LIC1 with 1:1 stoichiometry, or rather with 2:2 stoichiometry, since all the effectors identified to date form dimers. In this way, each effector could in principle tether two LIC1 subunits from a single dynein dimer or from two different dynein dimers bound simultaneously to the dynactin complex. In the case of Hook-family effectors, the two LIC1-binding sites are physically separated from one another, as suggested by our rotary shadowing EM analysis (Fig. 1h, i), whereas in CC1-Box-containing effectors the two binding sites occur on the same coiled-coil, i.e., adjacent to each other. Such structural differences, as well as differences in the affinities of the LIC1-effector interactions, may play a modulatory role, by forming dynein–dynactin-effector complexes of different affinities and characterized by different run lengths. In this regard, it is important to note that the dynein–dynactin-Hook3 complex displays a bimodal velocity distribution and faster velocities than the dynactin-BICD2 complex characterized by a single velocity

The activation of dynein processivity proceeds through a conformational change from an auto-inhibited so-called ‘phi particle’ state to a ‘parallel-heads’ state capable of binding microtubules upon complex formation with dynactin effectors. The auto-inhibited state is stabilized by inter-heavy chain inter−

**Methods**

**Proteins.** The cDNA encoding for human Hook1 (UniProt: Q9UJC3-1) and Hook3 (UniProt: Q86VS8-1) were purchased from Open Biosystems (Huntsville, AL). Constructs Hook111,146, Hook111,238, and Hook111,445 were cloned between BamHI and SalI sites of vector pMAL-c2x (NEB, Ipswich, MA), grown in E. coli BL21 (DE3) cells (Invitrogen, Carlsbad, CA), and harvested using the QuikChange site-directed mutagenesis kit (Agilent Technologies, Wilmington, DE). All the proteins were expressed in E. coli BL21 (DE3) cells (Invitrogen, Carlsbad, CA), grown in Terrific Broth medium at 37 °C until the OD600 reached a value of 1.5–2, followed by 16 h at 19 °C in the presence of 0.25 mM isopropyl β-D-thiogalactoside. The cells were collected by centrifugation, resuspended in 20 mM Tris, pH 7.0, 100 mM NaCl, 4 mM benzamidine hydrochloride, 1 mM PMSF, and 1 mM DTT and lysed using a Microfluidizer large-scale homogenizer (Microfluidics, Newton, MA). All the proteins were purified through an amylose affinity column according to the manufacturer’s protocol (NEB). The EMBL tag was removed by incubation with TEV protease overnight at 4 °C. The bacterial lysate was added to the amylose column and bound proteins were eluted with 1 mM DTT. The E. coli BL21 (DE3) cultures were transformed with the pMAL-c2x vector containing wild-type Hook1 or Hook3 with different point mutations and screened on ampicillin plates. The constructs were validated by sequencing. The final purification was done using the Ni-NTA affinity matrix (Qiagen) and the proteins were subjected to silver staining and/or Western blot analysis to confirm the purity of the eluted proteins. The purified proteins were subjected to size exclusion chromatography (Superdex 200 10/300 GL, GE Healthcare, Little Chalfont, UK) in 20 mM Tris, pH 7.0, 100 mM NaCl, 1 mM DTT. The DNA encoding for human LIC1 (UniProt: Q9Y6G9-1) was a generous gift from Ronald Vale (UCSF). Constructs LIC1FL, LIC11, LIC1-661, and LIC11,435 were

**Fig. 6 Model for cargo transport by dynein-dynactin-effector complexes.** Dynein is a 1.4 MDa homodimeric complex of two heavy chains, which each binds smaller subunits, including the intermediate chain, light intermediate chain, and three light chains. Dynactin is a ~1.0 MDa complex of more than 20 proteins, including an actin filament-like core of actin-related protein 1 (Arp1) subunits, capped at both ends by several subunits, and a ‘shoulder’ domain from which emerges the largest subunit, p150Glued, which projects ~50 nm and can bind microtubules directly to help initiate a processive run. Although dynein and dynactin bind directly to each other via the dynactin intermediate chain and the dynactin p150Glued subunit, they form a stable processive complex only in the presence of effector proteins, including Hook1/3,8,9, BICD2, Spindly, FIP3, and NIN/NINL10. These effectors are unrelated to each other in sequence and recruit different cargoes. Here we have demonstrated that independent of these differences, they all appear to interact with the same region of the dynein LIC1 subunit, which we named Helix-1 (or Effector-Binding Helix, EBH). The interaction involves the Hook domain in Hook-family effectors (left) or a coiled-coil segment in CC1-Box-containing effectors, such as BICD and Spindly (right). We have proposed here that the LIC1-effector interaction may help stabilize the ‘parallel-heads’ conformation thought to be necessary for dynein processivity.
amplified by PCR and cloned between the BamHI and Sall sites of a modified- vector pMAL-c2x that adds a C-terminal Strep-tag to the target protein. Point mutations F447A and F448A in LIC1433 were introduced using the QuikChange site-directed mutagenesis kit. Proteins were expressed and purified as described above, with one exception; after amylase affinity purification, the proteins were loaded onto a Strep-Tactin Sepharose column (IBA Lifesciences, Göttingen, Germany) and eluted after extensive washing with 3 mM desthiobiotin, 20 mM Tris, pH 7.0, 100 mM NaCl, and 1 mM DTT. To obtain the Helix-1 peptides, the cDNA encoding for LIC1433 (Fig. 2a) was cloned between the SapI and Sall sites of vector pTYB11 (NEB). Point mutations F447A and F448A were introduced using the QuikChange site-directed mutagenesis kit to obtain the mutant peptide Helix-1 R234A. Proteins were expressed as above, and purified on a chitin affinity column according to the manufacturer’s protocol (NEB), followed by auto- cleavage of the intein tag induced by incubation with 50 mM DTT overnight at 4 °C. The cleaved peptides were additionally purified on a Symmetry300 C8 reverse- phase column (Waters, Milford, MA) using an acetonitrile gradient of 0–90% (v/v) and 0.1% (v/v) trifluoroacetic acid.

The cDNA encoding for full-length Hook1 was codon optimized for expression in S9 cells and synthesized (Genscript Biotech, Piscataway, NJ). The gene was cloned between Sall and XbaI sites of a modified-vector pFastBac1, which adds a V5 epitope tag at the N terminus and a Strep-tag at the C-terminal of the target protein. The protein was expressed in Spodoptera frugiperda 9 (S9) cells using the Bac-to-Bac baculovirus expression system according to the manufacturer’s protocol (Invitrogen). The cells were collected by centrifugation, resuspended in lysis buffer (10 mM Na2HPO4, pH 7.4, 100 mM NaCl, 1 mM PMSF, 4 mM Benazmidine, 1 mM DTT, and 5% glycerol (v/v)) with addition of a protease inhibitors mixture (Roche).美发师. Washed cells were lysed in 250 mM NTA af.

Isothermal titration calorimetry

The dynein-driven motility of single Halo-Hook31–458 peptide at 4 °C for 1 h. Crystal were obtained at 20 °C using the hanging-drop method. The crystallographic drop consisted of a 1:1 (v/v) mixture of protein solution and well solution (1.44 M ammonium citrate tribasic, pH 6.25). The crystals were improved through consecutive rounds of micro-seedling. For data collection, the crystals were flash-frozen in liquid nitrogen from a cryo-solution consisting of addition of 30% (v/v) glycerol.

An x-ray diffraction dataset was collected at the Cornell High Energy Synchrotron Source (CHESS) beamline F1. The diffraction data were indexed and scaled using the program HKL2000. A molecular replacement solution was obtained with the program Phenix using PDB entry 5J8E (unbound Hook domain of Hook31). Model building and refinement were carried out with the programs Coot and Phenix. Figures were generated with the program PyMOL (Schrodinger, New York City, NY). Sequence alignments were carried out with the program MAFFT and visualized using ESPript. Data collection and refinement statistics are listed in Table 1.

Single-molecule motility assays

The motility of dynin-dynactin-Hook31–458 or dynein–dynactin-BICD2–572 complexes from cell extracts were tracked using TIRF microscopy. The motility assays were performed in flow chambers constructed with a glass slide and a silanized coverslip (PlusOne Repel Silane, GE Healthcare) coverslip, held together with double sided adhesive tape and vacuum grease to form a ~15 μl chamber. A 10-fold dilution of monoclonal anti-β-tubulin antibody (T5201, Sigma) was perfused into the chamber, which was subsequently blocked with 5% pluronics F-127 (Sigma-Aldrich, St. Louis, MO). Taxol-stabilized microtubules, labeled with HiLyte 488 or 647 (Cytoskeleton, Denver, CO) at a labeling ratio of 1:40, were flowed into the chamber and immobilized by interaction with anti-β-tubulin antibodies.

HeLa cells expressing Halo-tagged Hook31–552 or BICD2–572 were labeled with TMR-HaloTag ligand (Promega, Madison, WI) 18–20 h post-transfection. Cells grown in 10 cm plates to 70–80% confluence were then lysed in 100 μl lysis buffer (40 mM HEPES pH 7.4, 120 mM NaCl, 1 mM EDTA, 1 mM Mg-ATP, 0.1% Triton X-100, 1 mM PMSF, 0.01 mg/ml TAME, 0.01 mg/ml leupeptin and 1 μg/ml pepstatin-A). Cell lysates were then clarified by centrifugation at 17,000×g. Before flowing into the imaging chamber, the cell extracts were diluted in P12 buffer (12 mM PIPES, pH 6.8, 1 mM EGTA, 2 mM MgCl2, and 20 μM Taxol). The cell lysates were then further diluted in motility buffer (1× P12 buffer supplemented with 10 mM Mg-ATP, 0.3 mg/ml casein, 0.3 mg/ml bovine serum albumin, and 10 mM DTT) and an oxygen scavenging system (0.5 mg/ml glucose oxidase, 470 U/ml catalase and 15 mg/ml glucose) and flowed into the chamber to be imaged.

The dynine-driven motility of single Halo-Hook31–552 or Halo-BICD2–572 positive molecules was then examined in the absence or the presence of varying concentrations of the Helix-1 (10 μM) or Helix-1 (10 μM) peptide, added to the motility buffer immediately prior to the addition of the cell lysate. All the movies (4 frames/s) were acquired at room temperature using a Nikon TIRF system (Perkin Elmer, Waltham, MA) on an inverted Ti microscope equipped with a 100× objective and an ImageEM C9100-13 camera (Hamamatsu Photonics, Hamamatsu, Japan). Particle tracking was performed using the TrackMate plugin in the program Fiji.

Particle runs were tracked only if both the start and end of a run were observable over the course of the movie. Runs on microtubule bundles were excluded from analysis.

Data availability

Atomic coordinates and structure factor amplitudes for the crystal structure of the HOOK3-LIC1 complex were deposited with the Protein Data Bank (PDB) under accession code 6B9H. Other data and materials are available from the corresponding author upon reasonable request.

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References

1. Barlan, K. & Gelfand, V. I. Microtubule-based transport and the distribution, tethering, and organization of organelles. Cold Spring Harb. Perspect. Biol. 9, a025817 (2017).
2. Bonifacino, J. S. & Neeftes, J. Moving and positioning the endolysosomal system. Curr. Opin. Cell Biol. 47, 1–8 (2017).
3. Hoogenraad, C. C. Springer & Akhmanova, A. Bicaudal D family of motor adaptors: linking dynein motility to cargo binding. Trends Cell Biol. 26, 327–340 (2016).
4. Carter, A. P., Diamant, A. G. & Urnavicius, L. How dynein and dynactin transport cargos: a structural perspective. Curr. Opin. Struct. Biol. 37, 62–70 (2016).
5. McKenney, R. J., Huynh, W., Tanenbaum, M. E., Bhabbha, G. & Vale, R. D. Activation of cytoplasmic dynein motility by dynactin-cargo adapter complexes. Science 345, 337–341 (2014).
6. Schlager, M. A., Hoang, H. T., Urnavicius, L., Bullock, S. L. & Carter, A. P. In vitro reconstitution of a highly processive recombinant human dynein complex. Enb. J. 33, 1855–1868 (2014).
7. Sipliter, D. et al. BICD2, dynactin, and LIS1 cooperate in regulating dynein recruitment to cellular structures. Mol. Biol. Cell. 23, 4226–4241 (2012).
8. Olenick, M. A., Tokito, M., Boczkowska, M., Donnings, B. & Holzbaur, E. L. Hook adaptors induce unidirectional processive motility by enhancing the dynein–dynactin interaction. J. Biol. Chem. 291, 18239–18251 (2016).
9. Schroeder, C. M. & Vale, R. D. Assembly and activation of dynein–dynactin by the cargo adapter protein Hook3. J. Cell Biol. 214, 309–318 (2016).
10. Schroeder, C. M., Ostrem, J. M., Hertz, N. T. & Vale, R. D. A Ras-like domain in the light intermediate chain bridges the dynein motor to a cargo-binding region. eLife 6, e28257 (2017).
11. Luiro, K. et al. Interconnections of CLN3, Hook1 and Rab proteins link Batten disease material in this article are included in the article indicated otherwise in a credit line to the material. If material is not included in the original author(s) and the source, provide a link to the Creative Commons license and your intended use is not permitted by statutory material and in institutional affiliations.
12. Barlan, K. & Gelfand, V. I. Microtubule-based transport and the distribution, tethering, and organization of organelles. Cold Spring Harb. Perspect. Biol. 9, a025817 (2017).
13. Bonifacino, J. S. & Neeftes, J. Moving and positioning the endolysosomal system. Curr. Opin. Cell Biol. 47, 1–8 (2017).
14. Hoogenraad, C. C. Springer & Akhmanova, A. Bicaudal D family of motor adaptors: linking dynein motility to cargo binding. Trends Cell Biol. 26, 327–340 (2016).
15. Carter, A. P., Diamant, A. G. & Urnavicius, L. How dynein and dynactin transport cargos: a structural perspective. Curr. Opin. Struct. Biol. 37, 62–70 (2016).
16. McKenney, R. J., Huynh, W., Tanenbaum, M. E., Bhabbha, G. & Vale, R. D. Activation of cytoplasmic dynein motility by dynactin-cargo adapter complexes. Science 345, 337–341 (2014).
17. Schlager, M. A., Hoang, H. T., Urnavicius, L., Bullock, S. L. & Carter, A. P. In vitro reconstitution of a highly processive recombinant human dynein complex. Enb. J. 33, 1855–1868 (2014).
18. Sipliter, D. et al. BICD2, dynactin, and LIS1 cooperate in regulating dynein recruitment to cellular structures. Mol. Biol. Cell. 23, 4226–4241 (2012).
19. Olenick, M. A., Tokito, M., Boczkowska, M., Donnings, B. & Holzbaur, E. L. Hook adaptors induce unidirectional processive motility by enhancing the dynein–dynactin interaction. J. Biol. Chem. 291, 18239–18251 (2016).
20. Schroeder, C. M. & Vale, R. D. Assembly and activation of dynein–dynactin by the cargo adapter protein Hook3. J. Cell Biol. 214, 309–318 (2016).
21. Jordan, C. C. et al. Mammalian golgi 
22. Kardon, J. R. & Vale, R. D. Regulators of the cytoplasmic dynein motor. Curr. Opin. Cell Biol. 20, 467–477 (2011).
23. Zhang, K. et al. Cryo-EM reveals how human cytoplasmic dynein is auto-inhibited and activated. Cell 169, 1303–1314 (2017).
24. Jördens, L. et al. The Rab7 effector protein RILP controls lysosomal transport by inducing the recruitment of dynein–dynactin motors. Curr. Biol. 21, 1680–1685 (2011).
25. Johansson, M. et al. Activation of endosomal dynein motors by stepwise assembly of Rab7–RILP–p150Glue, ORP1L, and the receptor bIII spectrin. J. Cell Biol. 176, 459–471 (2007).
26. Tan, S. C., Scherrer, J. & Vale, R. B. Recruitment of dynein to late endosomes and lysosomes through light intermediate chains. Mol. Biol. Cell 22, 467–477 (2011).
27. Trooker, M., Mücke, N. & Surrey, T. Reconstitution of the human cytoplasmic dynein complex. Proc. Natl Acad. Sci. USA 109, 20895–20900 (2012).
28. Kardon, J. R. & Vale, R. D. Regulators of the cytoplasmic dynein motor. Nat. Rev. Mol. Cell Biol. 10, 854–865 (2009).
29. Hoogenraad, C. C. et al. Mammalian golgi-associated Bicaudal-D2 functions in the dynein–dynactin pathway by interacting with these complexes. EMBO J. 20, 4041–4054 (2001).
30. Bielaska, E. et al. Hook is an adapter that coordinates kinesin-3 and dynein cargo attachment on early endosomes. J. Cell Biol. 204, 989–1007 (2014).
31. Sano, H. et al. The microtubule-binding protein Hook3 interacts with a cytoplasmic domain of scavenger receptor A. J. Biol. Chem. 282, 7973–7981 (2007).
32. Horgan, C. P., Hanscom, S. R., Jolly, R. S., Futter, C. E. & McCaffrey, M. W. Rab11FIP3 links the Rab11 GTPase and cytoplasmic dynein to mediate transport to the endosomal-recycling compartment. J. Cell Sci. 123, 181–191 (2010).
33. Chowdhury, S., Ketcham, S. A., Schroer, T. A. & Lander, G. C. Structural organization of the dynein–dynactin complex bound to microtubules. Nat. Struct. Mol. Biol. 22, 345–347 (2015).
34. Urnavicius, L. et al. The structure of the dynein complex and its interaction with dynein. Science 347, 1441–1446 (2015).
35. Urnavicius, L. et al. Cryo-EM shows how dynein recruits two dyneins for faster movement. Nature 554, 202–206 (2018).
36. Grothjahn, D. A. et al. Cryo-electron tomography reveals that dynein recruits a team of dyneins for processive motility. Nat. Struct. Mol. Biol. doi:10.1038/s41594-018-0027-7 (2018).