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HYPOTHESIS

SUBJECT COLLECTION: CYTOSKELETON

Dynein light intermediate chains as pivotal determinants of dynein multifunctionality

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ABSTRACT

In animal cells, a single cytoplasmic dynein motor mediates microtubule minus-end-directed transport, counterbalancing dozens of plus-end-directed kinesins. The remarkable ability of dynein to interact with a diverse cargo spectrum stems from its tightly regulated recruitment of cargo-specific adaptor proteins, which engage the dynactin complex to make a tripartite processive motor. Adaptor binding is governed by the homologous dynein light intermediate chain subunits LIC1 (DYNC1LI1) and LIC2 (DYNC1LI2), which exist in mutually exclusive dynein complexes that can perform both unique and overlapping functions. The intrinsically disordered and variable C-terminal domains of the LICs are indispensable for engaging a variety of structurally divergent adaptors. Here, we hypothesize that numerous spatiotemporally regulated permutations of posttranslational modifications of the LICs, as well as of the adaptors and cargoes, exponentially expand the spectrum of dynein–adaptor–cargo complexes. We thematically illustrate the possibilities that could generate a vast set of biochemical variations required to support the wide range of dynein functions.

KEY WORDS: Cytoplasmic dynein, Light intermediate chains, Cargo selectivity, Adaptor, Posttranslational modification

Introduction

The cytoplasmic dynein 1 motor (referred to henceforth as dynein) is primarily responsible for microtubule minus-end-directed cargo transport in the cytoplasm of animal cells (Vale, 2003; Vallee et al., 2012). Dynein is a multi-subunit nanomotor built around a core of large dimeric heavy chains (HCs), which perform ATP hydrolysis to power motion and provide a molecular scaffold for assembling the intermediate chains (ICs), light intermediate chains (LICs) and light chains (LCs) into the dynein holocomplex (Pfister et al., 2006). Vertebrate cells contain ∼45–50 types of microtubule plus-end-directed kinesin motors, as compared to only a single dynein (Reck-Peterson et al., 2018; Vale, 2003). Thus, dynein has evolved to bind to and transport a large cargo spectrum to match that of the kinesins. The cargo binding versatility of dynein is beginning to be understood through the discovery of dynein adaptors, which help recruit the regulator complex dynactin and increase the processivity, speed, force generation and cargo spectrum of dynein (Olenick and Holzbaur, 2019; Reck-Peterson et al., 2018). In recent years, the dynein LIC subunits have emerged as critical determinants that directly engage with the adaptors (Celestino et al., 2019; Gama et al., 2017; Lee et al., 2018, 2020; Schroeder et al., 2014; Schroeder and Vale, 2016), which in turn engage with specific cargo subsets. In this Hypothesis, we posit that the structural and biochemical features of the adaptor-binding C-terminal domains (CTDs) of the LICs could be key conserved determinants of their adaptor-binding specificity, thus playing a defining role in dictating the cargo specificity and functional diversity of dynein.

The light intermediate chain subunits drive the functional diversity of dynein

The single invertebrate LIC has diverged into three (LIC1, LIC2 and LIC3, also known as DYNC1LI1, DYNC1LI2 and DYNC2LI1, respectively) in the vertebrates (Pfister et al., 2006). LIC1 and LIC2 are found in cytoplasmic dynein 1, whereas LIC3 assembles into cytoplasmic dynein 2 (which is confined to the cilioplasm) (Grissom et al., 2002; Hou et al., 2004). This article will focus on LIC1 and LIC2 (henceforth referred to as LICs), which are present in mutually exclusive dynein complexes (LIC1-dynein and LIC2-dynein) (Hughes et al., 1995; Tynan et al., 2000) that can perform both overlapping and unique cellular functions (Fig. 1). In mammalian cells during interphase, LIC1-dynein transports pericentrin to the centrosomes (Tynan et al., 2000), organizes Golgi vesicles at the nuclear periphery (Palmer et al., 2009) and governs the distribution of lysosomes and late endosomes more prominently than LIC2 (Tan et al., 2011). The Rab4a–LIC1 interaction on early endosomes helps recruit dynein to regulate their transport (Bielli et al., 2001). LIC1-dynein binds to SNX8-positive endosomes, whereas LIC2-dynein is recruited to SNX1- and SNX4-positive endosomes (Hunt et al., 2013). However, both LIC1-dynein and LIC2-dynein engage with FIP3 (also known as RAB11FIP3) to deliver endocytosed transferrin to the endosomal recycling compartment (ERC) (Horgan et al., 2010a,b; Palmer et al., 2009). A modified form of LIC1 associates with *Xenopus laevis* melanosomes to mediate their centripetal migration (Reilein et al., 2003), and LIC2-dynein binds to Par3 (also known as PARD3) to cortically tether microtubules and position the centrosomes in migrating human fibroblasts (Schmoranzer et al., 2009). During rat brain development, LIC1-dynein mediates apical nuclear migration in neural progenitors, governs the multipolar to bipolar transition in nascent neurons and controls glial-guided neuronal migration, whereas LIC2-dynein is involved in terminal somal translocation (Goncalves et al., 2019).

Co-depletion of both LICs has been shown to have drastic effects on mitotic functions, including mitotic progression, nucleus–centrosome attachment, kinetochore localization, spindle organization, chromosome congression and pole focusing, similar to HC depletion (Jones et al., 2014; Raaijmakers et al., 2013). Later studies have documented subtle but significant differences, showing that LIC2-dynein plays a greater role in several mitotic functions, including spindle orientation and spindle assembly checkpoint

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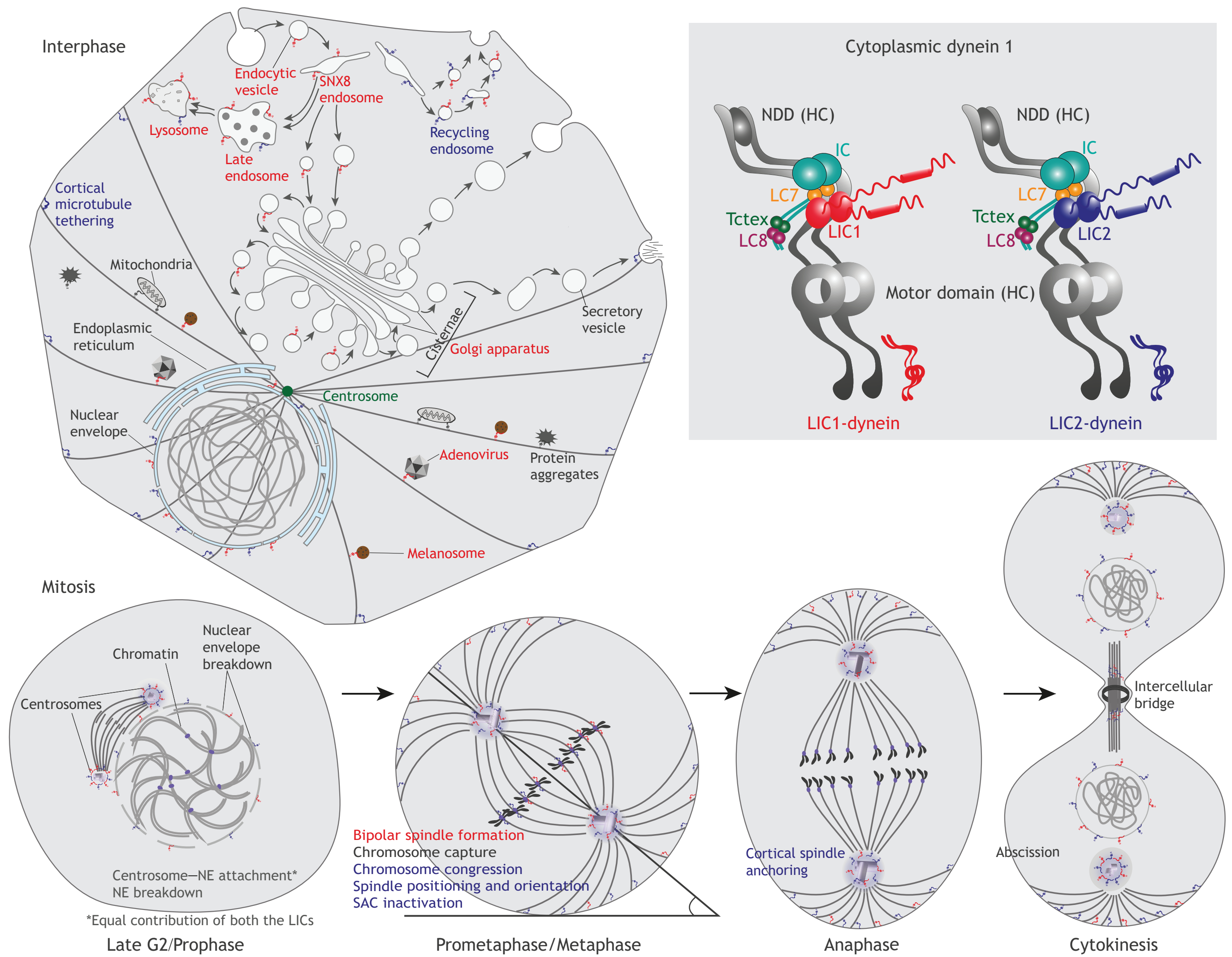

Fig. 1. Distinct functions of LIC1-dynein and LIC2-dynein. The vertebrate LIC homologues LIC1 (red) and LIC2 (blue) exist in mutually exclusive dynein complexes (see inset, top right). LIC1-dynein and LIC2-dynein complexes exert some distinct cellular functions, with either one or the other having a more prominent role. Cargoes are labelled in red or blue according to the LIC isoform involved and are shown attached to dynein complexes of the same colour. For cargoes in grey, no clear preference of either LIC is known. Distinct roles have been documented in both interphase (top left) and mitotic cells (bottom). NDD, N-terminal dimerization domain; NE, nuclear envelope.

(SAC) inactivation at metaphase (Mahale et al., 2016a,b). LIC2 depletion leads to elevated cortical levels but reduced polar levels of NuMA (also known as NuMA1), resulting in a more significant spindle misorientation compared with LIC1 depletion. Mitotic LIC2, but not LIC1, interacts specifically with 14-3-3ε, 14-3-3ζ and Par3 (Mahale et al., 2016a). The F-actin-bundling protein transgelin-2 interacts exclusively with mitotic LIC2-dynein and is required for spindle orientation by stabilizing the cortical LGN (GPSM2)–NuMA orientation machinery (Sharma et al., 2020). LIC2-dynein inactivates both the microtubule attachment-sensing and inter-kinetochore tension-sensing stages of the SAC, while LIC1-dynein inactivates only the former (Mahale et al., 2016b; Sivaram et al., 2009). LIC1, but not LIC2 is overexpressed in colorectal tumours and is required for colorectal carcinoma proliferation and migration in culture (Even et al., 2019). Thus, while the two homologous LIC subunits retain functional overlap, they have also sufficiently diverged to qualitatively and quantitatively fulfil several distinct dynein functions.

LIC–adaptor interactions increase the cargo selectivity and processivity of dynein

The dynein complex by itself exists in an autoinhibited ‘phi-dynein’ state, incapable of engaging with microtubules or cargoes (Torisawa et al., 2014; Zhang et al., 2017). Major conformational changes upon engaging an activating adaptor eventually lead to the formation of the active ‘parallel-head’ state, which interacts with dynactin to form a processive dynein–adaptor–dynactin tripartite complex on microtubules (Fig. 2) (Belyy et al., 2016; Elshenawy et al., 2019; Urnavicius et al., 2018; Zhang et al., 2017). Adaptor engagement is essential for imparting all essential attributes of an efficient motor–microtubule binding, speed, processive motility, force generation and cargo binding (Elshenawy et al., 2019; Grotjahn et al., 2018; Guo et al., 2016; McKenney et al., 2014; Schlager et al., 2010, 2014). Cryo-electron microscopy (cryo-EM), proteomic and biochemical data reveal multiple key interaction interfaces that optimize the architecture and function of the tripartite complex. In the first dynein–dynactin interface,

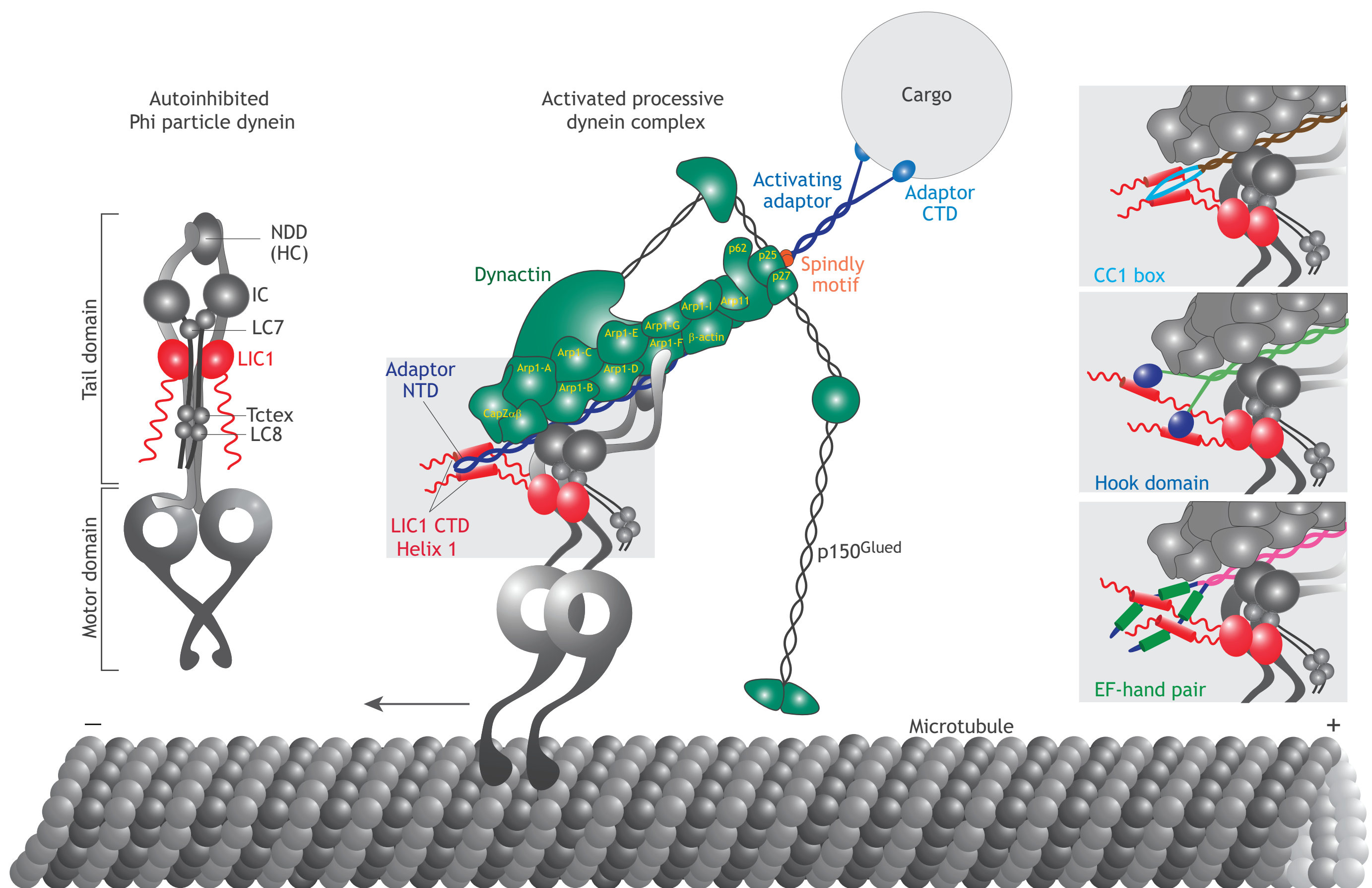

Fig. 2. Activation of dynein by adaptors. Left: dynein exists in an auto-inhibited phi particle form that is incapable of efficiently binding microtubules or dynactin and is virtually immotile. Middle: adaptor binding activates dynein to form the highly processive dynein–adaptor–dynactin tripartite complex, which can engage cargoes. All known activating adaptors contain a long, central coiled coil aligned along the dynein–dynactin interface (dark blue) and differ the most in their cargo-binding CTDs. Adaptors bind to helix 1 of the LIC CTD (in red) through any one of three conserved adaptor NTD domains: the CC1 box, Hook domain or EF-hand pair (see insets on the right). Arp1, actin-related protein 1 (also known as ACTR1A); Arp11, actin-related protein 11 (also known as ACRT10); CapZαβ, F-actin-capping protein heterodimer; NDD, N-terminal dimerization domain; p62, dynactin subunit 4 (DCTN4).

the HC tail domain binds to the Arp1 filament of dynactin and the IC subunit binds to the dynactin p150^{Glued} (DCTN1) subunit. The second interface, which regulates dynein–dynactin affinity and stoichiometry, forms through the intercalation of the long, coiled-coil region common to all adaptors, which runs parallel along the length of dynactin. The adaptor N-terminal domain (NTD) is positioned towards the barbed end of dynactin, while the variable CTD beyond the spindly motif [a short, conserved coiled-coil segment of the adaptor that contacts the p25 (DCTN5) or p27 (DCTN6) subunit of dynactin] engages with cognate cargoes. The third interface forms between the LIC CTD and the adaptor NTD; it likely modulates the net force, velocity and processivity of the tripartite complex and is indispensable for activating dynein (Celestino et al., 2019; Gama et al., 2017; Lee et al., 2018, 2020; Redwine et al., 2017; Urnavicius et al., 2018). Taken together, these studies reveal that divergent LIC-binding sites within various adaptor NTDs modulate the affinity and processivity of the ternary complex in an adaptor-specific manner.

All known activating adaptors can be categorized into three families based on the architecture of the interface formed by their respective adaptor NTDs and the LIC CTDs. Adaptors containing a CC1 box include BICD2 (Chowdhury et al., 2015; Urnavicius et al., 2015), BICDR1 (also known as BICDL1; Grotjahn et al., 2018; Urnavicius et al., 2018) and spindly (Gama et al., 2017; McKenney et al., 2014). Hook-domain adaptors include Hook1, Hook2 and Hook3 (Dwivedi et al., 2019; Olenick et al., 2016; Schroeder and Vale, 2016), as well as NuMA (Renna et al., 2020), although NuMA also contains an additional CC1-like box that engages with dynein LIC. Finally, EF-hand-containing adaptors include FIP3 (McKenney et al., 2014), ninein and ninein-like (Redwine et al., 2017), Rab45 (also known as RASEF) and CRACR2A (Wang et al., 2019). Other potential dynein adaptors are HAP1, TRAK1, TRAK2, RILP, JNK-interacting proteins (JIPs, also known as MAPK8IPs), girdin and daple, which interact with dynein and dynactin; however, their direct role in inducing dynein motility remains to be demonstrated (Reck-Peterson et al., 2018; Redwine et al., 2017). All known adaptors exist as dimers capable of engaging two LIC subunits, either from a single dynein (as is seen for BICD2) (Chowdhury et al., 2015; Urnavicius et al., 2015) or from two different dyneins (as is seen for BICDR1 and Hook3) (Grotjahn et al., 2018; Urnavicius et al., 2018) recruited simultaneously to dynactin. In CC1-box adaptors, the two LIC1 interaction sites lie adjacently on the same coiled coil, whereas they are distantly positioned in Hook-domain adaptors (Fig. 2, grey insets) (Lee et al., 2018). Remarkably, a conserved, short helix (helix-1) in the LIC CTDs binds to structurally unrelated adaptor NTD clefts through four conserved hydrophobic residues (Figs 2,4), making distinct intermolecular contacts within each interface, as observed from co-crystal structures and biophysical analyses of

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LIC1 CTD–adaptor NTD pairs representing all three adaptor families (Celestino et al., 2019; Lee et al., 2018, 2020). It is unknown how the LIC CTD positions its critical helix-1 in the correct register within the different adaptor clefts. Here, we propose how the properties of the LIC CTD itself could contribute significantly towards ensuring the pliability of the dynein–adaptor interface.

Structural principles underlying LIC–adaptor interactions

Secondary structure prediction analyses of representative vertebrate LICs suggest that the overall architecture of the LICs is well conserved (Fig. S1). The well-structured LIC NTD adopts a G-protein fold, with a series of conserved hydrophobic residues present in both LICs making close contacts with the HC tail (Schroeder et al., 2014; Urnavicius et al., 2018; Zhang et al., 2017). Human LIC1 and LIC2 share a high sequence identity (∼63%) and similarity (∼74%), especially within the NTD (Fig. S1). The functional separation of LIC1-dynein and LIC2-dynein (Fig. 1) is therefore likely to result mostly from the sequence divergence in its CTD (Fig. S1).

The largely unstructured LIC CTD has been implicated in adaptor binding (Celestino et al., 2019; Dwivedi et al., 2019; Lee et al., 2018, 2020; Schroeder et al., 2014). The LIC CTD exhibits characteristic features of an intrinsically disordered protein region (IDPR) (Oldfield and Dunker, 2014; Wright and Dyson, 2015) as it contains small, structured binding motifs (helices H1 and H2; Fig. 3A) while the flanking sequences remain disordered (linker L1 between the G-domain and H1; linker L2 between H1 and H2; Fig. 3A) (Celestino et al., 2019; Lee et al., 2018). Although H1 is the essential adaptor-binding structural motif (Celestino et al., 2019; Lee et al., 2018, 2020), increasing the length of the upstream L1 leads to an increased affinity for multiple adaptors (Celestino et al., 2019), a feature reminiscent of typical IDPRs (Oldfield and Dunker, 2014). The requirement for L1 is underscored by the compromised ability of any LIC1 CTD constructs that are devoid of L1 to bind most adaptors (except RILP), despite containing intact H1 and downstream sequences (Celestino et al., 2019). The largely flexible linkers could potentially adopt subtly different local conformations to optimally position H1 in the structurally unique hydrophobic interaction clefts of different adaptors. NMR analysis has provided evidence of additional interactions of multiple adaptors (Hook3, BICD2, spindly) with other LIC CTD sites neighbouring H1 (especially helix H2 and residues 418–421 in L1), albeit to different extents (Celestino et al., 2019), suggesting that the essential H1–cleft binding interface could be augmented and/or modulated by several flanking interactions.

There is well-documented precedence for a similar regulation of dynein function through the IC subunits. Each IC contains well-structured CTDs consisting of WD40 repeats that dock with the HC, while the relatively disordered and less conserved IC NTD recruits various binding partners, including dynein LCs (Hall et al., 2010; Makokha et al., 2002; Mok et al., 2001; Susalka et al., 2002), the regulators dynactin and NudE (also known as NDE1; Jie et al., 2017; McKenney et al., 2011; Vaughan and Vallee, 1995), as well as possibly several cargoes, including kinesin light chains 1 and 2 (Ligon et al., 2004) and huntingtin (Caviston et al., 2007), to the dynein complex. Similar to the LIC CTDs, the IC NTDs are typical IDPRs; they contain two helical binding motifs, SAH (required for NudE binding) and H2 (required for p150^{Glued} binding, along with SAH), flanked by the intrinsically disordered linkers L1 and L2 (Fig. 3A) (Barbar, 2012). The flexible linker L2 that connects H2 with the structured WD40-repeat domain of the IC CTD contains binding motifs for the dynein light chains LC7 (also known as Robl; DYNLRB1 and DYNLRB2 in mammals; Hall et al., 2010), LC8 (DYNLL1 and DYNLL2 in mammals; Makokha et al., 2002) and Tctex-1 (also known as DYNLT1; Mok et al., 2001), whose binding induces the acquisition of transient structure in these regions. Interestingly, the functionally analogous linkers L2 (of the IC NTDs) and L1 (of the LIC CTDs) bridge their respective helical interaction motifs with the HC-anchored structured domains and so provide multiple interaction surfaces to help recruit critical dynein binding partners. The IDPR-like structural pattern and mutually exclusive binding shown by the IC NTD (for different regulators and cargoes) (Barbar, 2012; Nyarko et al., 2012) and by the LIC CTD (for different adaptors) (Lee et al., 2020) suggests the possibility of a common evolutionary theme that has enabled these two key dynein subunits to optimally calibrate the motor's function.

LIC posttranslational modification – a key regulator of dynein's adaptor binding?

Various posttranslational modifications (PTMs) modulate the interactions and functions of dynein. HC phosphorylation redistributes dynein from the membrane to the cytosol (Dillman and Pfister, 1994; Lin et al., 1994). HC and IC phosphorylation cause a decrease in dynein-mediated microtubule engagement of lysosomes and endosomes (Runnegar et al., 1999). Phosphorylation of LC7 facilitates binding to ICs, which alters its cargo-binding properties (Tang et al., 2002), whereas phosphorylation of Tctex-1 and LC8 at specific sites facilitates their dissociation from the ICs without affecting interactions with other proteins (Song et al., 2007; Yeh et al., 2006). The motor ATPase activity of dynein drastically decreases upon dynactin-assisted phosphorylation of dynein LCs (Kumar et al., 2000).

Phosphorylation-mediated modulation of IC governs its biochemical interactions and cellular functions (Pfister, 2015; Pfister et al., 1996a,b). Multi-site phosphorylation in the flexible IC NTD generates multiple phospho-isoforms, which dictate binding partner selectivity. High-resolution NMR analysis has demonstrated that S84 phosphorylation, located in linker L2, selectively abrogates p150^{Glued} binding, but does not affect NudE binding, indicating that PTMs in the linkers flanking the binding interface (SAH and H2) can impart binding selectivity (Jie et al., 2017). Interestingly, this phosphorylation-dependent partner selectivity (NudE versus p150^{Glued}) is isoform-specific to IC-2C (encoded by *DYNC1I2*), but does not impact the IC-1A isoform (encoded by *DYNC1I1*), owing to the residue specificity of linker L1, as well the difference in length of linker L2 (Jie et al., 2017). Mitotic phosphorylation at the nearby site T89 enhances the binding of IC to ZW10 [a member of the kinetochore dynein receptor complex ROD (KNTC1)–ZW10–ZWILCH, known as RZZ] at the expense of p150^{Glued}, thus enabling dynein recruitment to the kinetochore (Bader et al., 2011; Whyte et al., 2008). Other phosphorylation events in the IC NTDs (for example, S80 of IC-1B, S87/T88 of IC-1B or S88/T89 of IC-2C) by various kinases, including extracellular signal–regulated kinases (ERK1 and ERK2, also known as MAPK3 and MAPK1, respectively) and glycogen synthase kinase-3β (GSK3β), in different cell types also modulate the functional specificity of dynein in an isoform-specific manner (Gao et al., 2015; Mitchell et al., 2012).

Human LIC1 and LIC2 contain numerous experimentally validated PTM sites revealed through proteomic studies (see the legend of Fig. 3 for PhosphoSitePlus database links; Hornbeck et al., 2015) for phosphorylation, ubiquitylation, monomethylation and acetylation (Fig. 3B,C; Fig. 4). The majority of the conserved PTMs appear to have emerged in the vertebrates (Fig. 4) and are clustered in

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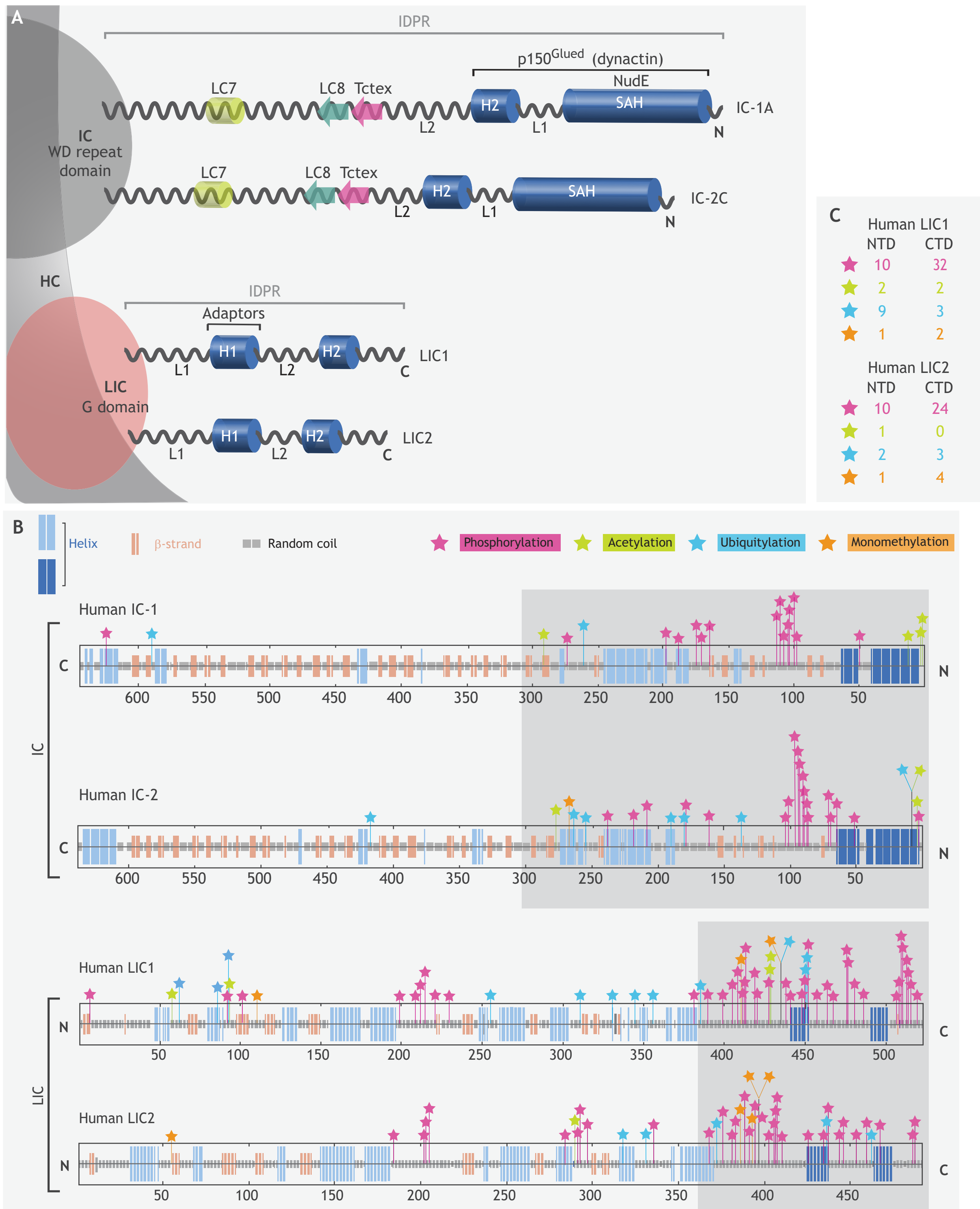

Fig. 3. See next page for legend.

the LIC CTDs, although some smaller clusters and scattered PTM sites exist in the NTDs. The number of phosphorylation sites is greater than the number of other PTMs (Fig. 3B,C). The role of CDK1-mediated LIC1 CTD phosphorylation in releasing dynein's interphase membranous cargoes, possibly to engage new mitotic cargoes, is well documented (Addinall et al., 2001; Dell et al., 2000).

The LICs incorporate significantly more phosphate than other dynein subunits during mitotic entry (Niclas et al., 1996). Multiple mitotic phosphoproteome studies show that virtually all of this phosphorylation is concentrated in the LIC CTDs, with more phosphorylation observed in LIC1 than in LIC2 (Daub et al., 2008; Dephoure et al., 2008; Olsen et al., 2010). The evolutionary

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Fig. 3. Posttranslational modifications could expand the functional diversity of the LICs. (A) Schematic representation of prototypical mammalian IC isoforms (IC-1A and IC-2C) and LIC isoforms (LIC1 and LIC2) anchored to the HC through their respective structured domains (WD-repeat domain of the IC CTDs, G-domain for the LIC NTDs). The largely unstructured IDPRs of both subunits protrude outward and share a similar organization, including two conserved helices each (blue) that are required for binding of dynein regulators. The varied lengths and/or sequences (see Fig. 4) of the flexible linkers, L1 and L2, could play crucial roles in dictating partner binding strength and/or specificity. (B) Secondary structure prediction of human IC and LIC isoforms (performed using the algorithms HNN, MLRC, DPM, DSC, PHD and PREDATOR at the NPSA server; https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_seccons.html) and distribution of their PTMs (as listed in the PhosphoSitePlus database; LIC1, https://www.phosphosite.org/uniprotAccAction?id=Q9Y6G9; LIC2, https://www.phosphosite.org/uniprotAccAction?id=O43237; IC1, https://www.phosphosite.org/uniprotAccAction?id=O14576; IC2, https://www.phosphosite.org/uniprotAccAction?id=Q13409). Dark blue helices represent the two crucial partner-binding helices of IC (H2, SAH) and LIC (H1, H2), as shown in A. Dark grey boxes represent the approximate IDPRs, in which PTMs are enriched. Numbers indicate amino acid positions. (C) The distribution of various PTMs in the NTDs and CTDs of the two human LICs, colour-coded as in B.

conservation of these sites across representatives from vertebrate phyla (Fig. 4) indicates their possible requirement for important cellular functions. Most of the PTM sites cluster within linkers L1 and L2 flanking the recognition motifs H1 and H2, which fits well with the general distribution of PTM sites observed in IDPRs (Oldfield and Dunker, 2014; Wright and Dyson, 2015). It needs to be empirically determined how these phosphorylation events could help in mitotic adaptor selection and cargo engagement.

In a manner analogous to the phosphorylation-based selective interactions shown by the IC isoforms, isoform-specific modifications of LIC1 and LIC2 could be an important mode of regulation of dynein function. For example, the LIC1-specific, protein kinase A (PKA)-mediated T213 phosphorylation that is located in an unstructured region of the LIC1 NTD switches dynein from the lysosome-associated adaptor RILP to the adenoviral capsid hexon protein upon infection; LIC2-dynein is unable to bind and transport the adenovirus, as LIC2 lacks this phosphorylation site (Scherer et al., 2014), demonstrating the cargo selectivity imposed by this isoform-specific phosphorylation of the LICs. The endogenous function of this LIC1-specific phosphorylation in uninfected cells is unclear. CDK1-mediated S207 phosphorylation in the same flexible region of LIC1 is required for metaphase-to-anaphase progression (Sivaram et al., 2009), whereas phosphorylation at the analogous S194 of LIC2 is required for maintaining centrosome integrity and enabling optimal microtubule nucleation to orient the spindle (Sharma et al., 2020). The S194A (phospho-deficient) mutant of LIC2 loses its ability to bind to the centrosomal microtubule nucleating factor γ-tubulin, which could cause reduced nucleation. Notably, mitotic S194 LIC2 phosphorylation modulates early mitotic functions, such as chromosome congression, pole-mediated microtubule nucleation, spindle orientation and timely anaphase onset, whereas the CTD phosphorylation (at S383 or S391) controls progression through cytokinesis (Sharma et al., 2020).

The broad, IDPR-like structural similarities between the LIC CTDs and IC NTDs are of particular interest. Similar to the important roles played by the linkers in the IC NTDs (which are IDPRs), the LIC linkers L1 and L2 could have important regulatory roles in adaptor binding. L1 shows a significant difference in primary sequence between the two LICs (Fig. 4) and a differential distribution of PTMs. For example, LIC1 L1, but not LIC2 L1 contains acetylation sites; conversely, LIC2 L1 harbours more monomethylation sites than LIC1 L1 (Fig. 3B, Fig. 4). Furthermore, a glycine-proline-rich stretch of twelve residues in the LIC1 L2, notably missing in LIC2 L2 (Fig. 4), could provide additional conformational flexibility, and along with unique PTMs (Fig. 3A, Fig. 4), could modulate the binding to specific adaptors. It is conceivable that the high number of conserved phosphorylation sites, together with other conserved PTMs in the LIC CTDs (Fig. 3B, Fig. 4), could have defining roles in selecting dynein adaptors and cargoes. The high conservation of the phosphorylation sites within the hydrophobic binding motif H1, or immediately flanking it (Fig. 4), suggests that these sites may play important roles. Interestingly, the hydrophobic adaptor clefts are also flanked by, or interspersed with, conserved acidic residues (glutamic acid, aspartic acid), which would create a negatively charged surface, for instance the CC1 box and EF-hand motif (Lee et al., 2020) or Hook domains (Schroeder and Vale, 2016). An attractive possibility is that mitosis-specific phosphorylation at these LIC CTD residues (Dephoure et al., 2008; Olsen et al., 2010) would impose strong electrostatic repulsion, possibly weakening or precluding the binding of certain adaptors, for instance those that predominantly recruit interphase cargoes. Interestingly, some of these H1 phosphorylation sites are specific to one isoform (Fig. 4), suggesting additional complexity imparted by isoform-specific modulation. This could be a potentially dynamic and reversible mechanism to determine adaptor specificity or switching.

Ubiquitylation, acetylation and methylation of lysine or arginine residues all regulate subcellular localization and/or interactions with other proteins (Bedford, 2007; Komander, 2009; Narita et al., 2019). Dynein subunits contain several non-degradative ubiquitylation sites (Kim et al., 2011). Inhibition of ubiquitylation results in excessive spindle pole accumulation of dynein during mitosis, indicating a possible effect of the ubiquitylation status of dynein subunits on its motility and/or its interaction with specific adaptors or cargoes (Monda and Cheeseman, 2018). The presence of multiple ubiquitylation sites on the ICs and the LICs, with some located close to the partner-binding helical motifs (Fig. 3B), suggests the possibility of ubiquitylation-mediated modulation of binding (Komander, 2009). All LIC CTD ubiquitylation sites are positioned close to or within H1 and H2. Although both LICs are ubiquitylated at conserved sites towards the end of H1, one site in each is conserved in an isoform-specific manner (K435 in LIC1, K461 in LIC2; Fig. 3B, Fig. 4), suggesting that these sites could modulate dynein interactions depending on the LIC isoform. Similarly, the acetylation sites are unique to LIC1 L1, whereas there are more monomethylation sites in LIC2 L1 (Fig. 4). Since linker L1 is required for optimal adaptor binding (Celestino et al., 2019), these PTM patterns could play important roles in adaptor specificity and/or affinity. However, these hypotheses merit experimental validation.

LIC subunits as crucial determinants of dynein functionality

The LIC CTDs are indispensable for adaptor and cargo engagement. The rapid discovery of new dynein adaptors has begun to unearth how dynein selects among multiple cargoes and performs its diverse array of cellular functions (Fig. 1). It is remarkable that a small, invariant region of the supple LIC CTD, the conserved helix H1, engages with structurally divergent adaptors and is able to mediate productive cargo transport. It is reasonable to postulate that the LIC CTD itself is modulated by spatiotemporally regulated PTMs, which enable it to rapidly switch between adaptors according to specific cellular requirements, thus creating an ensemble of functionally distinct dynein populations (Fig. 5). Subject to the

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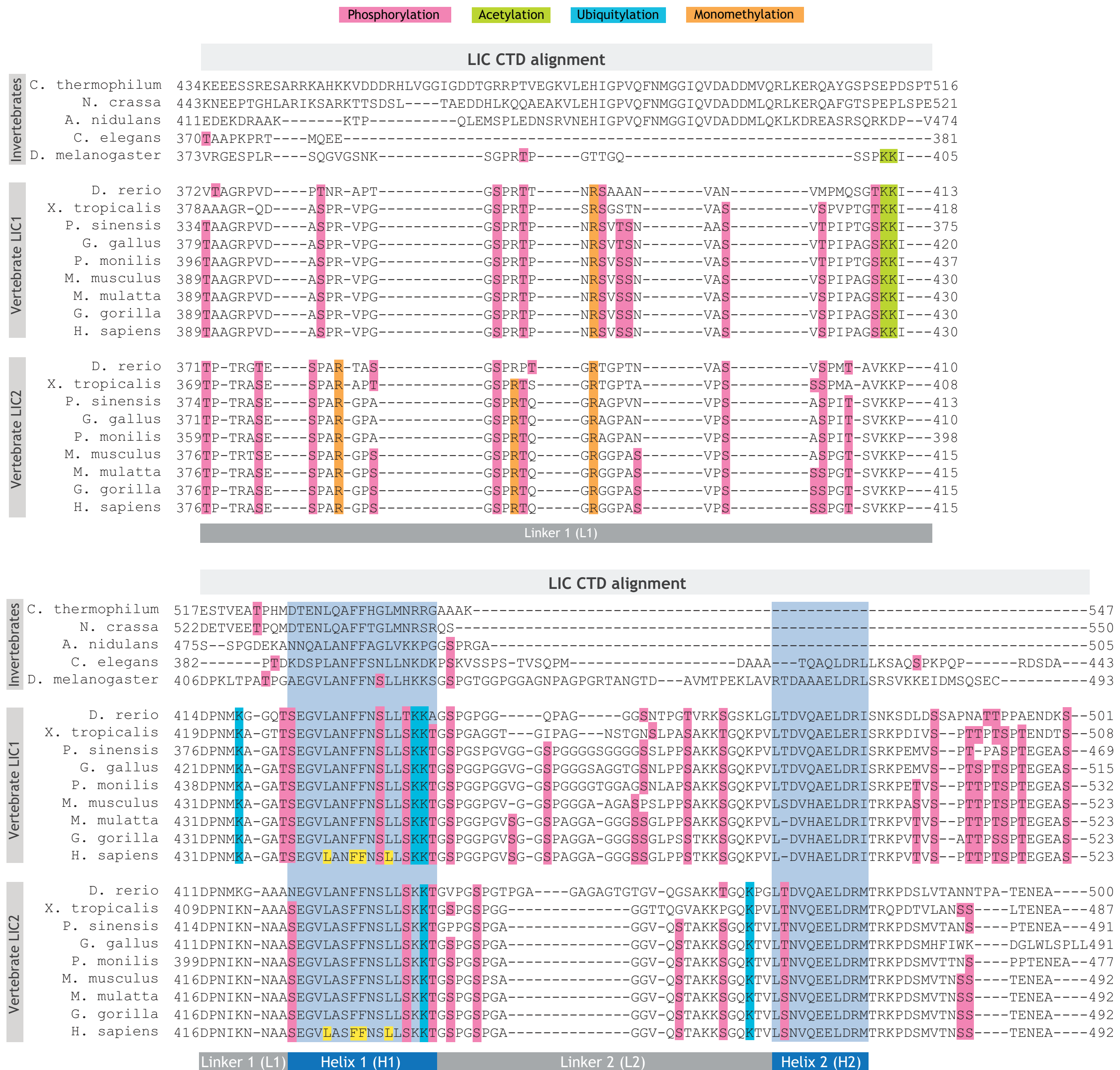

Fig. 4. Primary sequence alignment of the CTDs of LIC1 and LIC2. The sequence alignment shows several evolutionarily conserved isoform-specific PTMs, such as the acetylation and ubiquitylation sites preceding H1 that are unique to LIC1. Yellow boxes indicate the four conserved helix H1 residues involved in hydrophobic contacts with adaptor clefts. The evolutionary conservation of residues and PTMs indicates their functional importance, whereas isoform specificity within a species presents sites for differential functional modulation. Numbers indicate amino acid positions. *A. nidulans*, *Aspergillus nidulans*; *C. elegans*, *Caenorhabditis elegans*; *C. thermophilum*, *Chaetomium thermophilum*; *D. melanogaster*, *Drosophila melanogaster*; *D. rerio*, *Danio rerio*; *G. gallus*, *Gallus gallus*; *G. gorilla*, *Gorilla gorilla*; *H. sapiens*, *Homo sapiens*; *M. mulatta*, *Macaca mulatta*; *M. musculus*, *Mus musculus*; *N. crassa*, *Neurospora crassa*; *P. monilis*, *Patagioenas fasciata monilis*; *P. sinensis*, *Pelodiscus sinensis*; *X. tropicalis*, *Xenopus tropicalis*.

subunit composition of dynein being otherwise identical, the LICs could modulate dynein adaptor and cargo recruitment in two different ways. First, the two LICs, which occupy mutually exclusive dynein complexes and exhibit substantial sequence divergence in their cargo-binding CTDs, could form one important layer of selectivity. Second, adaptor selectivity could be imparted by conserved PTMs of the LICs, especially in their adaptor-binding CTDs (Fig. 5), which could help dictate the specificity and/or strength of binding. The presence of at least four types of largely conserved PTMs could by itself serve to regulate the engagement of adaptors. While this hypothesis needs experimental validation, it gains credence from the overall similarity with the flexible IC NTDs, whose site-specific phosphorylations have been conclusively demonstrated to regulate partner selection and dynein function (Gao et al., 2015; Jie et al., 2017; McKenney et al., 2011; Mitchell et al., 2012). These two layers of regulation could either operate in a mutually exclusive manner (in the case of isoform-specific modulation), or could act in combination to

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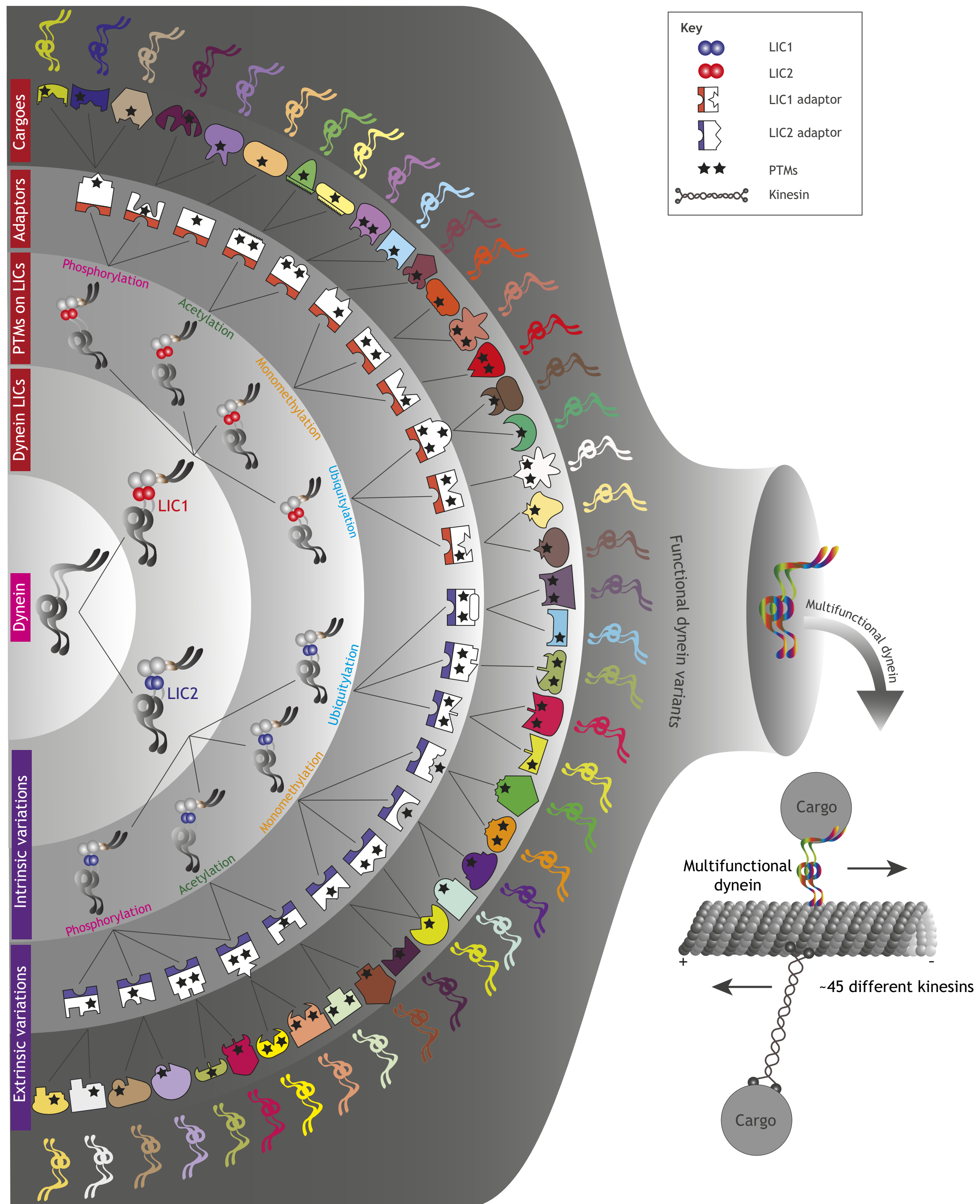

Fig. 5. Model for the possible LIC subunit-mediated expansion of the functional repertoire of dynein. The core subunits of cytoplasmic dynein (HC and IC) engage with either LIC1 or LIC2, each of which may exhibit one of a potentially vast number of PTM combinations, resulting in differential adaptor engagement. Similarly, PTMs on adaptors and cargoes might give rise to a diverse spectrum of functionally distinct dynein variants (multifunctional dynein) that match the repertoire of the ∼45 types of vertebrate kinesins. Some of the experimentally validated PTMs at each of these layers that modulate dynein function are discussed in the main text.

provide exquisite control of dynein partner specificity, as seen for the IC partners NudE and dynactin (Jie et al., 2017).

There is evidence that the interactions observed between recombinant LICs and adaptors may not always mirror those occurring *in vivo*, as suggested by analysis of cell lysates. For instance, the extent of binding to several adaptor NTDs (including those of BICD2, Hook3, spindly, RILP and FIP3) appears to be generally similar for recombinant LIC1 and LIC2 (Celestino et al.,

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2019); however, affinity precipitation experiments using cell lysates suggest that LIC1 and LIC2 have differential binding abilities for some adaptors (Redwine et al., 2017). For example, there is an approximately fourfold enrichment of LIC1-specific peptides interacting with Hook1 and Hook3 compared with LIC2-specific peptides. Conversely, an approximately threefold greater number of LIC2-specific peptides bind to the adaptor ninein (Redwine et al., 2017). Similarly, purified BICD2 NTD shows stronger binding to LIC1 than to LIC2 in lysates from rat embryonic brain (Goncalves et al., 2019). Thus, while many purified adaptors may have the inherent ability to bind to both purified LIC isoforms with approximately similar affinities, the physiologically relevant scenario might be more accurately represented in cell lysates owing to the presence of PTMs. Therefore, in order to fully understand the cargo selectivity and diversity of dynein, as well as its adaptor interactions, it is important to assess interactions in cells in addition to performing *in vitro* analyses with recombinant proteins.

Structurally distinct adaptors have been proposed to tune their interactions with the LIC CTD to achieve productive binding strength and motility triggered by cargo engagement. For example, the adaptor BICD2 is auto-inhibited, which can be relieved through conformational changes that are triggered by cargo engagement and that enable binding to dynein (Hoogenraad et al., 2003; McClintock et al., 2018; Noell et al., 2019; Sladewski et al., 2018); this conformational activation can be mimicked by specific adaptor mutations even in the absence of cargoes (Cui et al., 2020). Ca^{2+}-mediated activation of the adaptor CRACR2A is required for its binding to the LIC1 CTD, which induces dynein–dynactin motility (Lee et al., 2020; Wang et al., 2019). PTMs of the adaptors could additionally contribute to such fine-tuning and cargo selectivity to cater to dynein's functional diversity. For example, the mitotic adaptor spindly undergoes farnesylation at C602 in its cargo-binding CTD, which allows it to bind to ROD (Mosalaganti et al., 2017), while phosphorylation at two sites upstream (S594 and T600) have been proposed to further enhance this interaction, enabling it to serve as a kinetochore receptor for dynein and dynactin (Holland et al., 2015; Moudgil et al., 2015). In addition, the recently identified adaptor NuMA requires deubiquitylation before it can interact with dynein to promote bipolar spindle formation (Yan et al., 2015), and dephosphorylation (at T2055) to enable optimal binding with cortical dynein for the coordination of proper spindle orientation in metaphase and spindle elongation in anaphase (Kotak et al., 2013). Notably, several adaptor-specific phosphopeptides have been reported in studies investigating the interactomes of several adaptor NTDs (for BICD2, Hook1, ninein and ninein-like) (Redwine et al., 2017), as well as in whole-proteome phosphoproteomic screens (for Hook-1, -2 and -3, as well as BICD2) (Daub et al., 2008; Dephoure et al., 2008). Similarly, PTMs on cargoes could be required for adaptor binding. For example, S72 phosphorylation on the endosomally anchored Rab7a GTPase is required for binding to its effector (and dynein adaptor) RILP to mediate dynein-dependent endosomal transport towards lysosomes (Hanafusa et al., 2019). Taken together, a combination of PTMs on LICs, adaptors and cargoes could enable dynein to engage with a vast cargo spectrum in a spatiotemporally regulated manner, while at the same time only requiring a limited number of modulators.

Conclusions and perspectives

The vast array of cellular functions for dynein requires that any underlying biochemical modifications are finely-tuned and regulated in a precise and rapid manner. Such a degree of regulation is made possible by a spatiotemporal engagement with activating adaptors, which undergo crucial interactions with the flexible, PTM-enriched LIC CTD. Here, we postulate a model that positions the LIC subunits as crucial determinants of the multiple distinct dynein functions. Dynein has evolved to retain a largely invariant core identity, while still achieving remarkable functional versatility, which could be considerably further expanded through the numerous possible combinations of PTMs (Fig. 5). The vast array of possible PTM combinations in the LIC CTDs could provide the variety required to fine-tune the chemistry, conformation and interactions of the LIC CTD helices in an adaptor-specific manner (Fig. 3B, Fig. 4). The unstructured regions flanking H1 and H2 could provide the conformational flexibility, possibly facilitated or enhanced by PTMs, to accommodate the crucial H1-binding motif of diverse adaptors and allow for a dynamic switching between cargoes throughout the cell cycle. PTMs of the LICs could have evolved to complement the innate differences between the variable hydrophobic binding clefts presented by different adaptor families, in addition to the tunable adjustments in the adaptor, thus achieving productive transport of a number of diverse cargoes using a single motor. Dissecting the molecular mechanisms by which the LIC CTD may be calibrated by its plethora of PTMs would be challenging but certainly worthwhile, as it could help us to understand, and potentially devise strategies to modulate, dynein function with a high degree of precision.

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Competing interests
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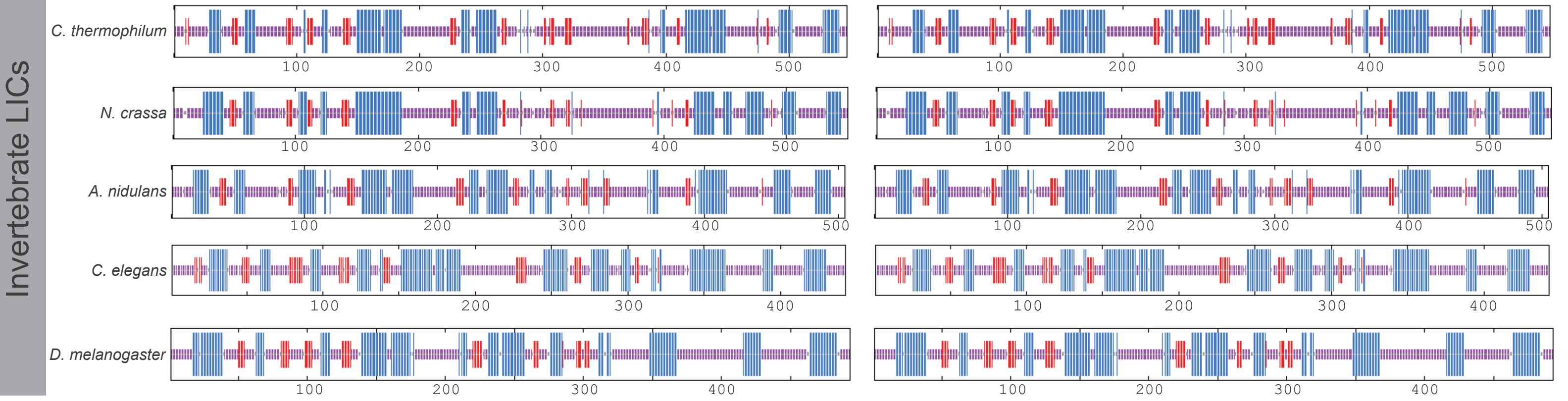
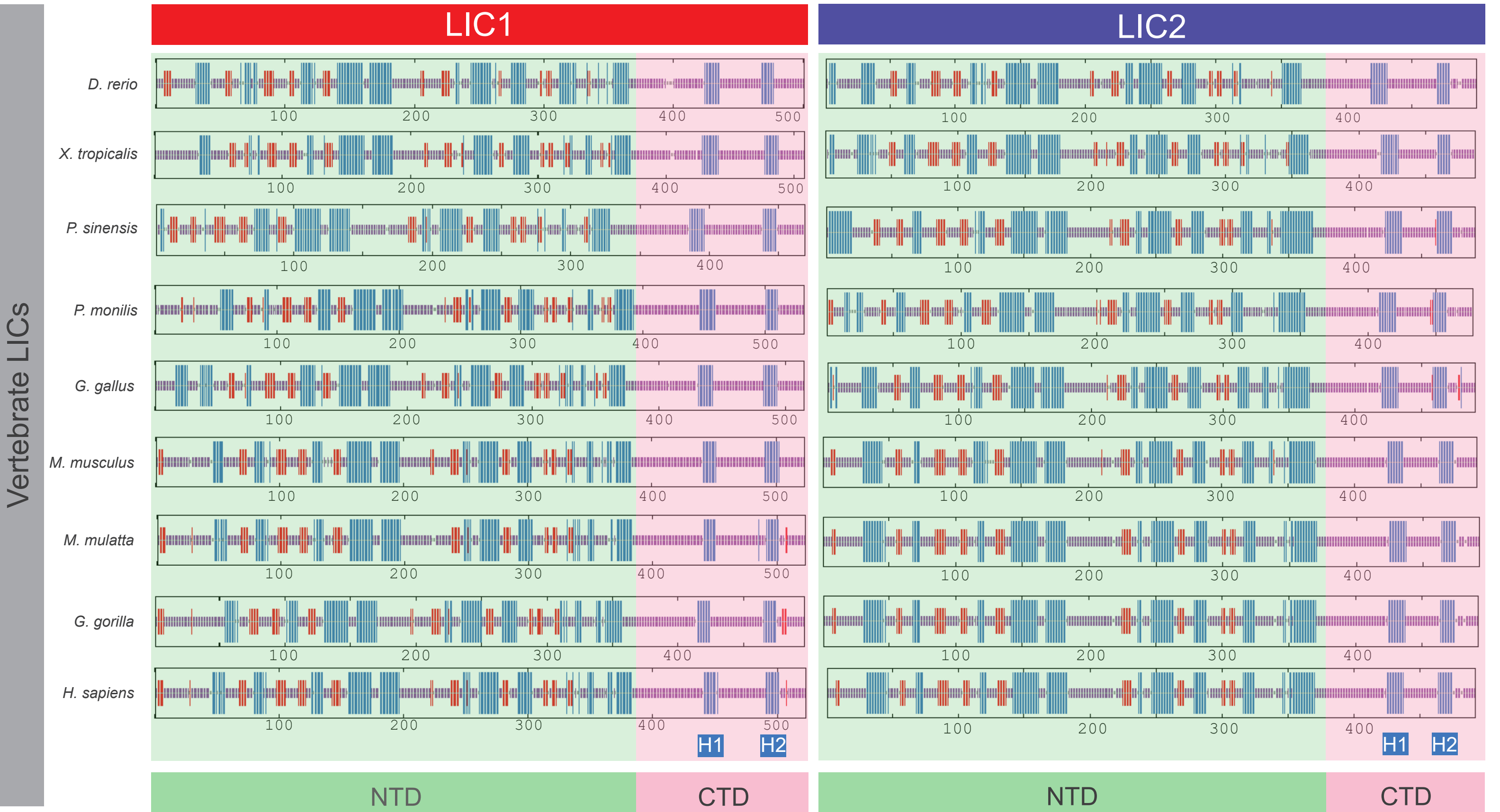
A





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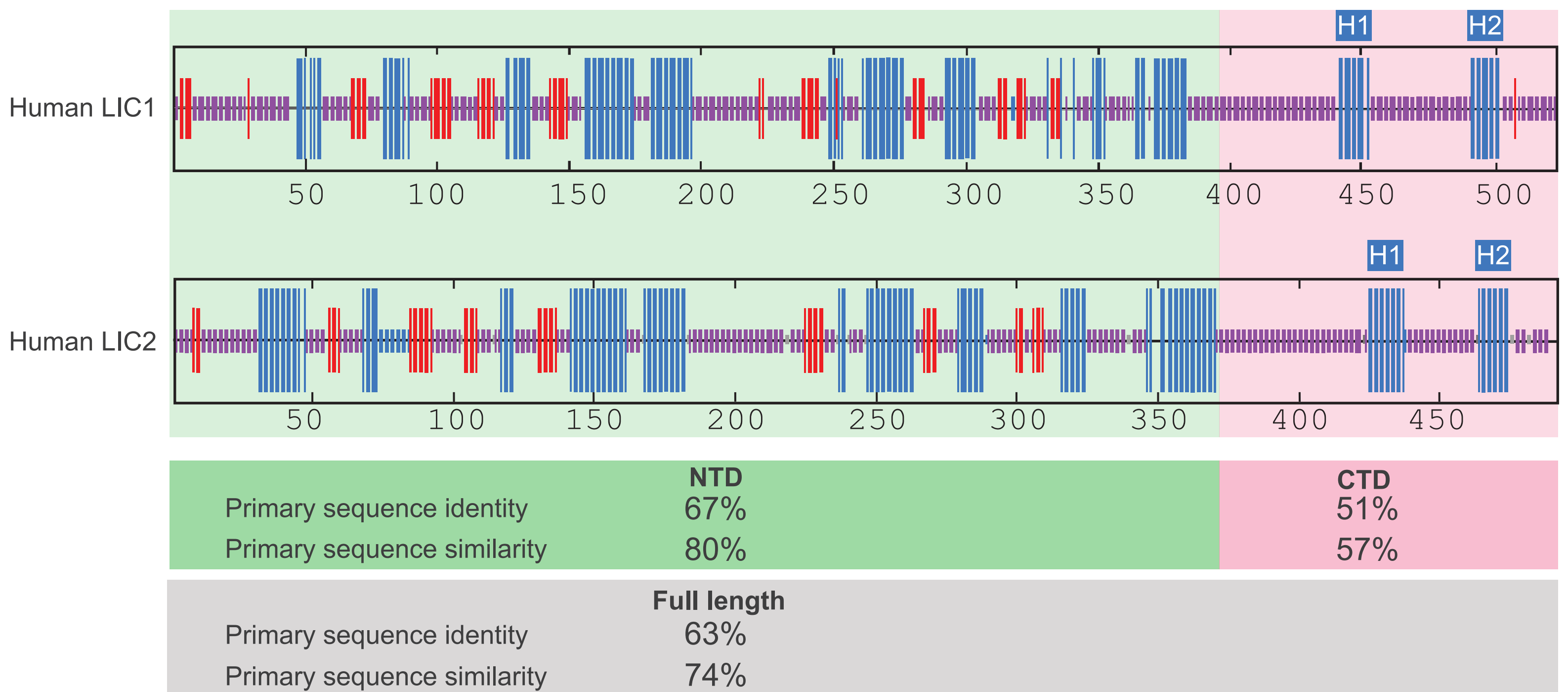

	NTD	CTD
Primary sequence identity	67%	51%
Primary sequence similarity	80%	57%

	Full length
Primary sequence identity	63%
Primary sequence similarity	74%

Fig. S1: The conserved structural features of the dynein LICs. (A) Schematic secondary structure alignment of the indicated LIC subunit primary sequences (obtained from NCBI) from both invertebrates and vertebrates. Predicted helices (blue), β-turns (red) and unstructured random coil (purple) regions are based on consensus secondary structure prediction on the NPSA server (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_seccons.html), performed using the algorithms HNN, MLRC, DPM, DSC, PHD and PREDATOR here and in Fig. 3B. (B) Despite showing similar overall structures, including the two conserved CTD helices H1 and H2, human LIC1 and LIC2 have a higher primary sequence divergence in their largely unstructured, cargo-binding CTDs.