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The cytoplasmic dynein transport machinery and its many cargoes

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Abstract | Cytoplasmic dynein 1 is an important microtubule-based motor in many eukaryotic cells. Dynein has critical roles both in interphase and during cell division. Here, we focus on interphase cargoes of dynein, which include membrane-bound organelles, RNAs, protein complexes and viruses. A central challenge in the field is to understand how a single motor can transport such a diverse array of cargoes and how this process is regulated. The molecular basis by which each cargo is linked to dynein and its cofactor dynactin has started to emerge. Of particular importance for this process is a set of coiled-coil proteins — activating adaptors — that both recruit dynein–dynactin to their cargoes and activate dynein motility.

Lissencephaly
Derived from Greek for 'smooth brain'; a spectrum of developmental disorders characterized by defective neuronal migration and the resulting lack of brain folds and grooves.

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The microtubule cytoskeleton is responsible for long-distance movements and spatial organization of intracellular vesicles, organelles and large protein-containing and RNA-containing complexes in many eukaryotic cells. Microtubules are polarized structures with a minus end and a plus end. In interphase cells, microtubule plus ends are typically located near the periphery. Minus ends originate from microtubule organizing centres (MTOCs), which are often located close to the nucleus but can also be found at other locations in the cell. In mitosis, microtubules reorganize to form the spindle, with the microtubule minus ends focused at the two poles. The molecular motors, dyneins (minus-end-directed) and kinesins (primarily plus-end-directed), are responsible for many microtubule-based functions. Notably, plants and some algae lack dynein genes in their genomes.

Dyneins were first discovered as the motors that drive flagellar beating in *Tetrahymena pyriformis*[1]. Subsequently, two dynein isoforms were found that are responsible for movement in the cytoplasm (cytoplasmic dynein 1) and cilia (cytoplasmic dynein 2)[2–5]. This Review will focus on cytoplasmic dynein 1 (subsequently referred to as dynein). Dynein is an essential gene in a number of organisms, including *Drosophila melanogaster* and mice[6,7], and mutations in the dynein transport machinery have been linked to neurological diseases. These include neurodegenerative disorders such as the Parkinson disease-like Perry syndrome, spinal muscular atrophy-lower extremity predominant (SMA-LED), hereditary motor neuron disease and Charcot-Marie-Tooth disease. They also include neurodevelopmental diseases such as lissencephaly, as well as other malformations of cortical development and intellectual disabilities[8].

Remarkably, a single dynein functions in the cytoplasm in animal cells, which is in contrast to the ~40 kinesins that perform related functions. This suggests dynein uses a different strategy to interact with its cargoes compared with kinesin. Here, we address how a single dynein transports such a diversity of cargoes. We first discuss the structure of the dynein-based transport machinery. We then describe the main cargoes of dynein, which we group under four categories: membranes, RNAs, proteins and viruses. We have limited this Review to interphase functions of dynein, as its mitotic and meiotic roles have been covered elsewhere[9].

The dynein transport machinery

After the discovery of dynein, it became clear that other factors were required for its activity. A high-molecular-weight complex, later named dynactin, was found to be necessary for vesicle movement along microtubules[10,11]. However, the two complexes only interacted weakly in vitro[12]. This interaction was shown to become stronger in the presence of an N-terminal domain of protein bicaudal D homologue 2 (BICD2), BICD2-N, which is predominantly coiled-coil[12]. BICD2 is the human homologue of bicaudal D (BicD), which was originally identified as a polarity factor in *D. melanogaster*[13]. Recent studies demonstrated that BICD2-N dramatically activates the motility of isolated dynein–dynactin complexes to move long distances in vitro[14,15]. Additional coiled-coil-containing proteins have also been shown to activate dynein–dynactin motility (TABLE 1). We refer to these coiled-coil proteins as activating adaptors. These proteins have the dual property of both activating motility and linking dynein–dynactin to their cargoes. Collectively, these results suggest the dynein transport machine consists of the dynein complex, the dynactin complex and a coiled-coil-containing activating

Table 1 | **Activating adaptors and candidate activating adaptors for dynein–dynactin**

Activator or candidate activator	Evidence	Cargo
Confirmed activating adaptors (active in in vitro motility assays)		
BICD2	Reconstituted motility[14,15] and relocation assay[225]	• COP1-independent Golgi-to-ER vesicles[58] • Golgi vesicles[57] • Nuclear pore complexes[134]
BICDL1	Reconstituted motility[16]	RAB6 vesicles[61]
SPDL1	Co-IP motility[14]	Kinetochore[228]
HOOK1	Lysate motility and relocation assay[227]	RAB5 early endosomes[70]
		Clathrin-independent cargoes[229]
HOOK3	Reconstituted motility[24], co-IP motility[14], lysate motility and relocation assay[227]	• RAB5 early endosomes[70] • Golgi[230]
NIN	Reconstituted motility[98]	Unknown
NINL	Reconstituted motility[98]	MICAL3 and RAB8A containing vesicles[63]
RAB11FIP3	Co-IP motility[14]	Recycling endosomes[28]
Candidate activating adaptors		
BICD1	Co-IP[231]	• COP1-independent Golgi-to-ER vesicles[58] • Microtubule arrays[231]
BICDL2	Homology to BICD proteins[61]	RAB13 vesicles[61]
HOOK2	Homology to Hook proteins	• Spermatid intramanchette trafficking[232] • Centrosomal proteins[175]
CCDC88A	Co-IP[98]	Unknown
CCDC88B	Co-IP[233]	Secretory lysosomes (lytic granules)[233]
CCDC88C	Co-IP[98]	Unknown
NUMA	Co-IP[234]	Minus ends of microtubules in the spindle[132,235]
TRAK1	Co-IP[111]	Mitochondria[111]
TRAK2	Co-IP[111]	Mitochondria[111]
HAP1	Co-IP[75,76,208]	Many membrane cargoes[75]

Each activator or candidate activator is listed along with its cargoes. Cargoes are defined by their reliance on dynein or dynactin for movement or localization. We have not included known activator interacting proteins where there is not yet evidence for dynein–dynactin involvement for their trafficking. BICD, protein bicaudal D homologue; BICDL1, BICD family-like cargo adapter 1 (also known as BICDR1); CCDC88A, girdin; CCDC88B, coiled-coil domain-containing protein 88B; CCDC88C, daple; co-IP, co-immunoprecipitation; COP1, E3 ubiquitin-protein ligase RFWD2; ER, endoplasmic reticulum; HAP1, huntingtin-interacting protein 1; HOOK, protein Hook homologue; MICAL3, [F-actin]-monooxygenase MICAL3; NIN, ninein; NINL, ninein-like; NUMA, nuclear mitotic apparatus protein; RAB, RAS-related protein; RAB11FIP3, RAB11 family-interacting protein 3; SPDL, Spindly; TRAK, trafficking kinesin-binding protein.

adaptor (such as BICD2) (FIG. 1; TABLE 1). Below, we provide an overview of these three central components, as well as two additional regulators, LIS1 (also known as PAFAH1B1 or platelet-activating factor acetylhydrolase IB subunit-α) and Nudel, a small protein family comprised of two related proteins NDE1 (nuclear distribution protein nudE homologue 1) and NDEL1 (nuclear distribution protein nudE-like 1). Both LIS1 and Nudel associate with the dynein complex and are required for many functions of dynein. Beyond activating adaptors, other connections between dynein and its cargoes have been shown to be involved in dynein transport (BOX 1).

Structure of dynein

Human dynein is a 1.4 MDa complex composed of six different polypeptides, all of which are present in two copies (FIG. 1a). There is a single dynein heavy chain (DHC) (encoded by *DYNC1H1*) and two isoforms of each of the other components: the intermediate chains (DIC; encoded by *DYNC1IC1* and *DYNC1IC2*), the light intermediate chains (DLIC; encoded by *DYNC1LI1* and *DYNC1LI2*) and the three dynein light chain (DLC) families: Roadblock (Robl; encoded by *DYNLRB1* and *DYNLRB2*), LC8 (encoded by *DYNLL1* and *DYNLL2*) and Tctex (encoded by *DYNLT1* and *DYNLT3*).

The DHC is 4,634 amino acids long and contains a C-terminal motor domain and an N-terminal tail domain. Starting from the N terminus of the DHC, the tail contains a dimerization domain (residues 1–200), followed by an extended region made up of nine helical bundles (residues 201–1420)[16–18] (FIG. 1b). The last helical bundle of the tail joins the motor domain, which consists of the linker (itself also made of helical bundles), a ring of six AAA+ domains and a C-terminal domain. The motor domain binds to its track via a microtubule-binding domain (MTBD) at the end of a coiled-coil stalk that emerges from the AAA+ ring[19,20]. Dynein moves along microtubules by coupling ATP-induced conformational changes in the AAA+ ring with bending and straightening of the linker[21].

AAA+ domains
Domains that are a highly conserved ATPase fold.

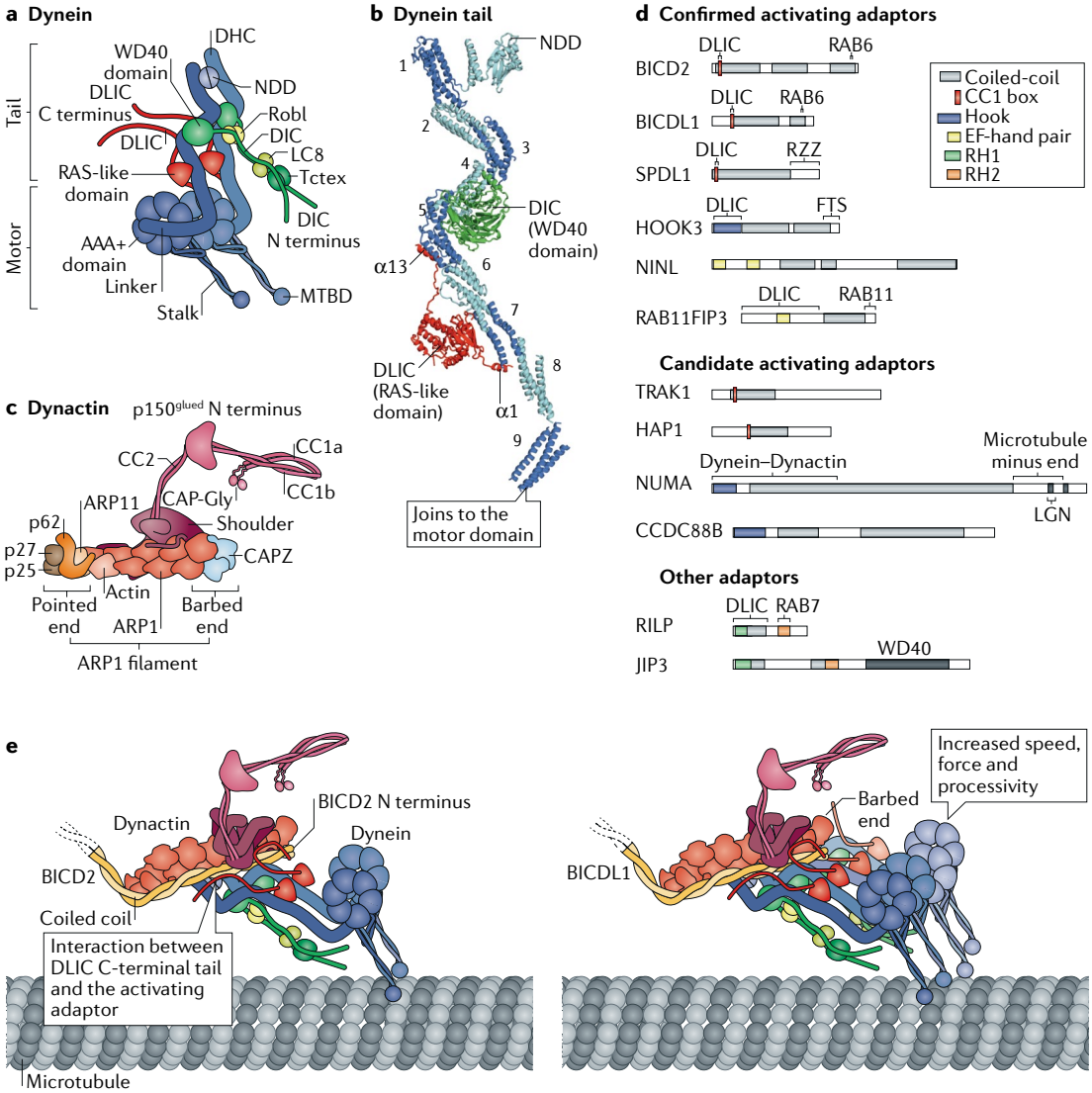


Fig. 1 | **The dynein transport machinery. a** | Cartoon of cytoplasmic dynein 1. The two dynein heavy chains (DHCs) are linked together by an N-terminal dimerization domain (NDD) and have a C-terminal motor domain with a microtubule-binding domain (MTDB) at the end of a long antiparallel coiled-coil stalk. The dynein intermediate chains (DIC) have extended N termini that bind dimers of the dynein light chains Roadblock (Robl), LC8 and Tctex. The dynein light intermediate chain (DLIC) has an extended C terminus. **b** | Structure of the dynein tail[16,18] (PDB accession numbers: 6F1T and 5NVU) showing the NDD and nine helical bundles (1–9; neighbouring bundles are shown in different shades of blue for distinction) in the DHC and depicting interactions of DHC with the DIC and DLIC. The DIC WD40 domain binds bundles 4 and 5, whereas the RAS-like domain of the DLIC binds to bundles 5 and 7, using its N-terminal and C-terminal helices (α1 and α13). The DIC and DLIC extended termini are not shown. The structure of helical bundles 8 and 9 is approximate. **c** | Dynactin is built around a filament of eight actin-related proteins (α-centractin (ARP1)). At the barbed end are capping proteins (F-actin capping protein CAPZ). At the pointed end is an actin monomer, another actin-related protein (actin-related protein 3B (ARP11)) and a complex of three proteins (p62, p27 and p25). The shoulder domain binds the filament via extended N-terminal peptides of p50 dynamitin. The p150 component extends from the shoulder, containing stretches of coiled coils (CC2, CC1b and CC1a). At the N terminus of p150 are the basic and CAP-Gly domains that can interact with microtubules. **d** | Domain structure of known and candidate activating adaptors (see also TABLE 1). Reported sites of interactions are indicated above each cartoon. Key shows domains identified in the literature or by InterPro. **e** | Dynein–dynactin–activator complexes on microtubules. The activating adaptors protein bicaudal D homologue 2 (BICD2) or BICD family-like cargo adapter 1 (BICDL1; also known as BICDR1) run along the dynactin filament and recruit the DHCs. BICD2 preferentially recruits one dynein dimer (left), whereas BICDL1 recruits two dimers (right). CCDC88B, coiled-coil domain-containing protein 88B; FTS, AKT-interacting protein (also known as AKTIP); HAP1, huntingtin-interacting protein 1; HOOK3, protein Hook homologue 3; JIP3, c-Jun-N-terminal kinase-interacting protein 3; LGN, G-protein-signalling modulator 2; NINL, ninein-like; NUMA, nuclear mitotic apparatus protein; RAB, RAS-related protein; RAB11FIP3, RAB11 family-interacting protein 3; RH, RILP homology; RILP, RAB-interacting lysosomal protein; RZZ, Rod–ZW10–Zwilch complex; SPDL1, Spindly; TRAK1, trafficking kinesin-binding protein 1.

Box 1 | **Other connections between dynein and its cargo**

In addition to the activating adaptors or candidate activating adaptors (TABLE 1), a number of other links between dynein–dynactin and its cargoes have been reported. Here, we present some prominent examples.

RAB-interacting lysosomal protein
RAB-interacting lysosomal protein (RILP) is required for dynein–dynactin recruitment to RAS-related protein 7 (RAB7) lysosomes[34]. Because RILP interacts with the HOPS complex, it may be involved in linking dynein–dynactin to RAB7 (FIG. 3c). Purified RILP co-precipitates with purified dynein light intermediate chain 1 (DLIC1), suggesting a direct interaction with the complex[23,35].

Huntingtin and huntingtin-associated protein 1
Huntingtin (HTT) and huntingtin-associated protein 1 (HAP1) associate with membrane vesicles[75] and are transported in neurons along microtubules[207]. HTT is linked to signalling endosomes[74] and autophagosomes[105]. HTT and HAP1 knockdown decreases retrograde movement of autophagosomes[105]. HTT binds purified dynein[75], suggesting a direct interaction that does not require dynactin. HAP1 co-immunoprecipitates with dynactin component p150 from brain lysate[76,208]. HAP1 contains a coiled-coil domain and has sequence similarity to trafficking kinesin-binding protein (TRAK) and BICD family-like cargo adapter 1 (BICDL1; also known as BICDR1)[60] (FIG. 1d; TABLE 1), raising the possibility that it is a BICD-like activating adaptor.

c-Jun N-terminal kinase-interacting proteins
c-Jun N-terminal kinase-interacting proteins (JIPs) are implicated in the motility of dynein–dynactin cargoes. JIP1 depletion inhibits retrograde transport of amyloid precursor protein[209] and autophagosomes[106] in neurons. JIP3 co-immunoprecipitates with dynactin and co-localizes with dynein and dynactin on vesicles in neurons[33]. JIP1 lacks coiled coils, whereas JIP3 contains two short stretches of coiled coil (FIG. 1d). JIP4 is closely related to JIP3 and shares the same domain architecture.

Ankyrin 2
Mice with ankyrin 2 (AnkB) deletion show reduction in the speed of fast axonal transport of early endosomes, lysosomes, mitochondria and synaptic vesicles. AnkB binds the lipid phosphatidylinositol 3-phosphate (PI3P) on membrane cargoes and directly contacts the pointed-end complex of dynactin[85].

Sorting nexins
Sorting nexins (SNXs) are a large family of membrane-associated proteins that contain a lipid binding PX domain. Affinity-tagged SNX5 and SNX6 co-precipitate with dynein and/or dynactin from human cell lysates[210,211].

Spectrin
Spectrin is a peripheral membrane protein that co-immunoprecipitates with dynactin[87]. While a two-hybrid screen reported an interaction between spectrin and α-centractin (ARP1)[212], the structure of the dynein–dynactin–BICD2 complex shows little fully exposed ARP1 available for binding[17].

Snapin
Snapin is a SNARE interacting protein, which is part of the biogenesis of lysosome-related organelles complex 1 (BLOC-1)[213]. It has been implicated in the retrograde movement of late endosomes and suggested to be a dynein adaptor[86]. Snapin is required for dynein to localize to late endosomes, and glutathione-S-transferase (GST)–snapin co-precipitates with dynein and dynactin components from brain lysate[86]. However, because these in vitro experiments were done in the absence of other BLOC-1 complex members, they should be revisited.

Direct cargo binding to dynein
In addition to its interaction with activating adaptors, the DLICs can form direct interactions with cargoes, such as the adenoviral hexon protein[35], pericentrin[214] and partitioning defective 3 homolog (PAR3)[215]. The dynein light chains (DLCs) LC8 and Tctex bind motifs found in many proteins, leading to the idea that these DLCs directly recruit proteins to dynein. However, because these motif binding sites on the DLCs bind to the dynein intermediate chain (DIC) N terminus, the current consensus is that this is not the case[216]. Many of the DLC binding motifs likely recruit LC8 or Tctex for dynein-independent functions, such as dimerization. However, there is some evidence for cargoes connecting to dynein via DLCs, including some viruses[182], rhodopsin[217,218] and the Rho guanine nucleotide exchange factor 2 (ARHGEF2)[219]. Additional structural studies will be required to determine if peptides from these proteins can bind to DLCs that are already in complex with the DIC N-terminal peptides; NMR mapping experiments suggest that this is possible[219,220].

The DIC contains a WD40 domain that binds the DHC helical bundles 4 and 5 and an extended ~230 residue N-terminus. The extended N-termini of both DICs are held together by dimers of DLCs. Tctex and LC8 dimers bind toward the N-terminal end of the extended region, wheras the Robl dimer binds closer to the WD40 domains. Robl also docks on the WD40 domain of one of the DICs. The DLIC contains a RAS-like domain that contacts DHC helical bundles 6 and 7 (REFS[16,18]). The DLIC is further anchored onto the DHC by N-terminal and C-terminal helices (α1 and α13, respectively) that span out and contact DHC helical bundles 8 and 5, respectively (FIG. 1b). The two DLICs have extended ~130-residue C termini (FIG. 1a), which contain two α-helices (α14 and α15)[22]. The DLIC C termini contact activating adaptors[22–24], and in some cases may also provide a direct link to cargo (BOX 1).

Structure of dynactin

The 1.1 MDa dynactin complex is composed of 23 subunits (11 different polypeptides; FIG. 1c)[25]. Its central feature is a short actin-like filament, which contains eight copies of the actin-related protein α-centractin (ARP1) (encoded by *ACTR1A* and *ACTR1B*) and one copy of β-actin (encoded by *ACTB*). The filament is capped at the barbed end by the F-actin capping protein CAPZ (encoded by *CAPZA1* or *CAPZA2* and *CAPZB*) and at the pointed end by another actin-related protein, ARP11 (encoded by *ACTR10*). Three other proteins, p62 (encoded by *DCTN4*), p27 (encoded by *DCTN6*) and p25 (encoded *DCTN5*), bind ARP11 to form a pointed-end complex. A shoulder domain sits on the filament near the barbed end. It is composed of two copies of p150^{glued} (p150; encoded by *DCTN1*), four copies of p50 dynamitin (p50; encoded by *DCTN2*) and two copies of p24 (encoded by *DCTN3*). Extended peptides corresponding to the N terminus of p50 emerge from the shoulder and wrap around one side of the filament. The C terminus of p150 is buried in the shoulder, and the N terminus forms a flexible extension with two stretches of coiled coil (CC1 and CC2) interrupted by a globular domain. CC1 contains two halves, CC1a and CC1b, which form a hairpin structure. At the extreme N terminus of p150 are the CAP-Gly and basic domains, which have been implicated in microtubule binding[26].

WD40 domain
A structural domain formed from WD40 repeats, themselves composed of approximately 40 amino acids and often ending in tryptophan (W), followed by aspartic acid (D).

SNARE
Proteins that are anchored to either donor or acceptor membranes, mediating fusion between distinct membranes.

BLOC-1
A multisubunit protein complex that contributes to membrane tubulation, which is important for sorting and organelle biogenesis in the endolysosomal system.

RAS-like domain
A protein domain with sequence similarity to the GTPase domain of RAS.

Barbed end
The end where myosin can be seen protruding when actin filaments are decorated with myosin motor domains and visualized by electron microscopy; similar nomenclature is used to refer to the equivalent end of the α-centractin (ARP1) minifilament in dynactin.

Pointed end
The end where myosin cannot be seen protruding when actin filaments are decorated with myosin motor domains and visualized by electron microscopy; similar nomenclature is used to refer to the equivalent end of the α-centractin (ARP1) minifilament in dynactin.

EF hands
A helix–loop–helix protein structural domain that often confers a protein with calcium-binding ability.

β-propellers
A protein structural domain characterized by four to eight wedge-shaped β-sheets arranged similarly to the blades on a propeller.

Activating adaptors

At the time of writing, eight activating adaptors have been shown to promote long distance movement of dynein–dynactin complexes in vitro (FIG. 1d; TABLE 1). There are no sequence motifs that are common to all of these activating adaptors. Instead, the common features are the presence of a long (>200 residues) coiled coil, a binding site for the DLIC C terminus[23,24,27] and a binding site for proteins (for example, RAS-related protein 6 (RAB6), RAB11, the Rod–ZW10–Zwilch (RZZ) complex and AKT-interacting protein (FTS; also known as AKTIP)) that link the adaptors to their cargoes (FIG. 1d).

Activating adaptors contain at least three different types of binding sites for the DLIC C terminus. BICD2, the BICD-related protein BICD family-like cargo adapter 1 (BICDL1; also known as BICDR1) and the kinetochore binding adaptor SPDL1 (Spindly) likely bind the DLIC via a motif referred to as the 'CC1 box' in their coiled coil[27]. The CC1 box contains an AAxxG sequence (where x denotes any amino acid). By contrast, protein Hook homologue 3 (HOOK3) and HOOK1 use a small Hook domain, which is located N-terminal to their coiled coils[24]. The penultimate helix (α14) in the DLIC C terminus is the contact site for BICD2, SPDL1 and HOOK3[22]. The activating adaptor RAB11 family-interacting protein 3 (RAB11FIP3) also binds the DLIC C terminus. The exact site of interaction is not yet known, but the region of RAB11FIP3 (residues 2–435)[28] that interacts with the DLIC contains a pair of EF hands. Interestingly, the same type of domain is also found in the activating adaptors ninein (NIN) and ninein-like (NINL), although these proteins have not yet been shown to bind the DLIC. All of the activating adaptors are known or predicted to be dimers.

Cryo-electron microscopy (cryo-EM) structures have been solved for dynein and dynactin in complex with three different activating adaptors (BICD2, BICDL1 and HOOK3)[16,17]. These cryo-EM maps are at medium (BICDL1) to low (BICD2, HOOK3) resolution in the regions around the activating adaptors but are sufficient to reveal the main ways in which the activating adaptors bring dynein and dynactin together. In all cases, a ~250 residue coiled coil of the activating adaptor runs along the length of the dynactin filament (FIG. 1e). Their N termini lie close to the barbed end of the dynactin filament, and their C termini contact the pointed end complex. The coiled coils of the activating adaptors interact with dynactin slightly differently, especially towards the pointed end. This is consistent with the lack of conserved motifs in the coiled coils of different activating adaptors. Interestingly, BICD2, HOOK3 and BICDL1 can all recruit two dynein dimers at a time[16,29], although for BICD2, the recruitment of a second dynein dimer appears to be less efficient[16]. The second dynein lies next to the first along the dynactin filament (FIG. 1e). These interactions, mediated by activating adaptors, recruit dynein to dynactin so that the individual DHCs bind in grooves between dynactin filament subunits[16,17]. Importantly, these interactions induce large conformational changes in dynein that align its motor domains so that both MTBDs can bind microtubules[18]. This likely underlies how activating adaptors increase the ability of dynein to move over long distances. The ability to recruit two dyneins to one dynactin further enhances this effect[16,29].

Dynein and dynactin also interact via the N terminus of the DIC contacting CC1 of p150 (REF.[30]). This interaction may reinforce the interactions described above. Alternatively, a recent report suggested that this DIC–p150 interaction inhibits dynein[31], raising the possibility that binding of an activating adaptor disrupts this interaction.

The activation of dynein includes a number of steps in addition to binding activating adaptors. Dynein in isolation can exist in an inhibited form, referred to as the 'Phi (ϕ)-particle' (REF.[32]), which not only binds weakly to microtubules but is also unable to bind dynactin and activating adaptors[18]. The mechanism by which the ϕ-particle opens up is not yet known.

In addition to the known activating adaptors, there are a number of possible candidate activating adaptors (FIG. 1d; TABLE 1). These proteins contain long coiled coils and co-immunoprecipitate with both dynein and dynactin. Sequence analysis suggests many of these putative activating adaptors have domains that bind the DLIC, although as of yet, this has not been directly tested. Trafficking kinesin-binding protein 1 (TRAK1), TRAK2 and huntingtin-interacting protein 1 (HAP1) contain the CC1 box motif[27], whereas nuclear mitotic apparatus protein (NUMA), girdin (CCDC88A), coiled-coil domain-containing protein 88B (CCDC88B) and daple (CCDC88C) all contain Hook domains (FIG. 1d).

An interesting question is whether RAB-interacting lysosomal protein (RILP) and c-Jun-N-terminal kinase-interacting protein 3 (JIP3) are activating adaptors. Similar to known activating adaptors, both proteins can interact with dynein and dynactin[33,34], with RILP directly binding to the DLIC[23,35]. While RILP and JIP3 both have regions of coiled coil, they are not long enough to bind to dynein and dynactin in the same way as other activating adaptors (FIG. 1d). They may therefore fall into a category of nonactivating adaptors (BOX 1) that serve as links between the dynein complex and cargo without the activation function.

Dynein regulation by LIS1 and Nudel

Two other central regulators of dynein are LIS1 and Nudel. They have been linked to dynein function genetically in many organisms (reviewed recently in REF.[36]). LIS1, a dimer of two β-propellers, binds directly to the motor domain of dynein at two distinct sites[37,38]. Nudel proteins are coiled-coil-containing proteins that interact with the DIC and LC8, as well as with LIS1 (REFS[39–41]). There is evidence that Nudel may tether LIS1 to dynein[38,40]. The molecular mechanism of LIS1-mediated regulation appears to be complex as in vitro experiments have revealed a range of LIS1 functions, including decreasing[38,42,43] and increasing velocity of the dynein motor[37,44,45]. Based on experiments using *Saccharomyces cerevisiae* dynein, the effects LIS1 exerts on dynein depend on the nucleotide state at its third AAA+ domain[37]. The function of Nudel also appears to be complex as it can both enhance[38,46] and oppose

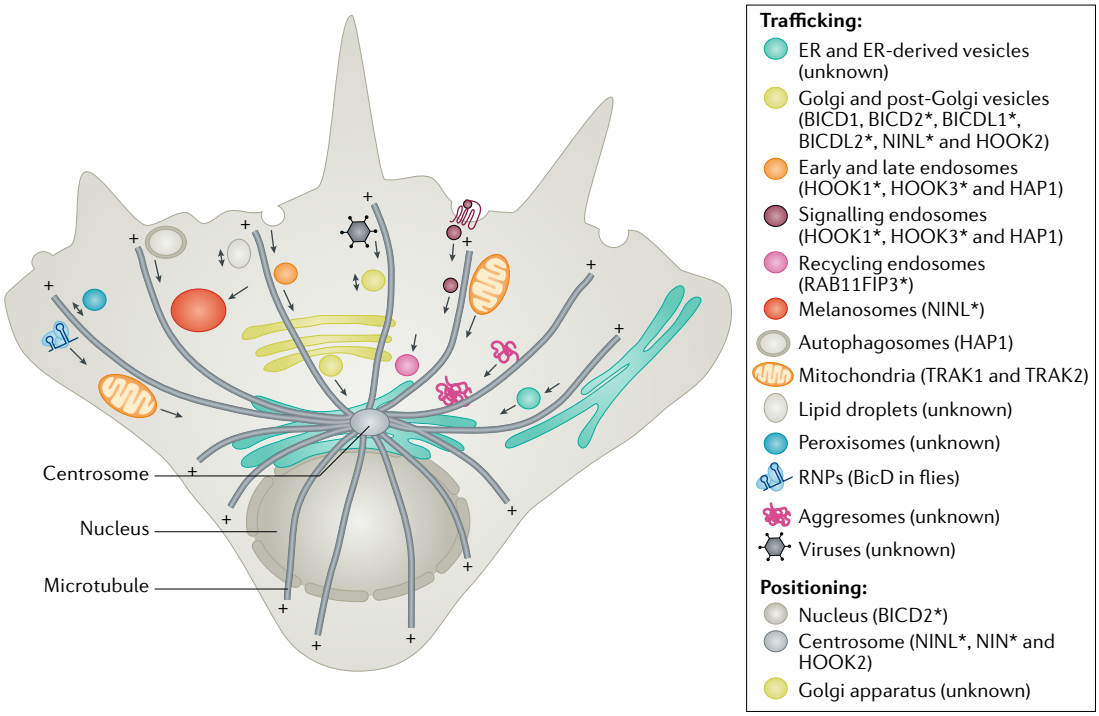


Fig. 2 | **Many cargoes of dynein and their activating adaptors.** A highly stylized schematic of a cell depicts many of the dynein cargoes discussed in this Review. Some cargoes are actively trafficked along microtubules, while for others the role of dynein is to position them. Activating adaptors (marked with a star) and candidate activating adaptors are listed where there is some experimental evidence that they are involved in transport or positioning. BicD, bicaudal D; BICD, protein bicaudal D homologue; BICDL1, BICD family-like cargo adapter 1 (also known as BICDR1); BICDL2, BICD family-like cargo adapter 2; ER, endoplasmic reticulum; HAP1, huntingtin-interacting protein 1; HOOK, protein Hook homologue; NIN, ninein; NINL, ninein-like; RAB11FIP3, RAB11 family-interacting protein 3; RNPs, ribonucleoproteins; TRAK, trafficking kinesin-binding protein.

LIS1 function in vitro[42,43]. LIS1 — probably in complex with Nudel proteins — has been implicated in multiple cellular processes, including localizing dynein to microtubule plus ends, initiating cargo transport and supporting the ability of dynein to transport high-load cargoes (reviewed recently in REF.[36]).

Dynein cargoes

The remainder of this Review focuses on the wide range of cargoes that dynein transports (FIG. 2). Studies with dextrans suggested that, whereas 500 kDa molecules can diffuse freely across a cell, complexes larger than 2 MDa are confined and effectively immotile[47]. Thus, cargoes for dynein are typically large objects, such as organelles and ribonucleoprotein (RNP) or protein complexes. Dynein can also act while it is anchored at the cell cortex, where it can act as a tether and/or generate pulling forces. Although many of these cargoes move bidirectionally owing to the interplay between dynein and kinesin, here we focus on dynein-based motility. For each cargo, we describe its physiological role and discuss the evidence for the involvement of dynein in its dynamics. We also describe the current state of knowledge for how each cargo is linked to dynein–dynactin, highlighting the role of known or candidate activating adaptors (FIGS 1d,2; TABLE 1). Our focus will be on vertebrates, although results from other organisms, such as flies and filamentous fungi, are also discussed.

Cell cortex
The cytoplasmic face of the plasma membrane.

Membrane cargoes

There are many discrete membrane-bound compartments in eukaryotic cells. Some of these are marked by small GTPases of the RAB family. RABs bind effector proteins to direct trafficking processes, including membrane tethering, fusion and movement mediated by molecular motors[48]. Disrupting dynein function (BOX 2) alters the cellular localization or motile properties of many of these membrane compartments.

Endoplasmic reticulum. The endoplasmic reticulum (ER) is a meshwork of membranes consisting of tubules and sheets. Tubules are particularly dynamic, and their movements require dynein as they are inhibited by overexpression of the dynactin component p50, which is a classic method for disrupting dynein–dynactin function[49] (BOX 2). Similarly, overexpression of p50 showed that dynein–dynactin is responsible for transporting ER membranes in neuronal dendrites[50].

Dynein also moves vesicles originating from the ER, which is spread throughout the cell, to the cell centre. These vesicles coalesce to form the ERGIC (endoplasmic reticulum Golgi intermediate compartment), a precursor compartment of the Golgi apparatus. Evidence for this comes from visualization of a secreted viral protein that exits the ER and moves rapidly to the centre of the cell. This movement is disrupted by

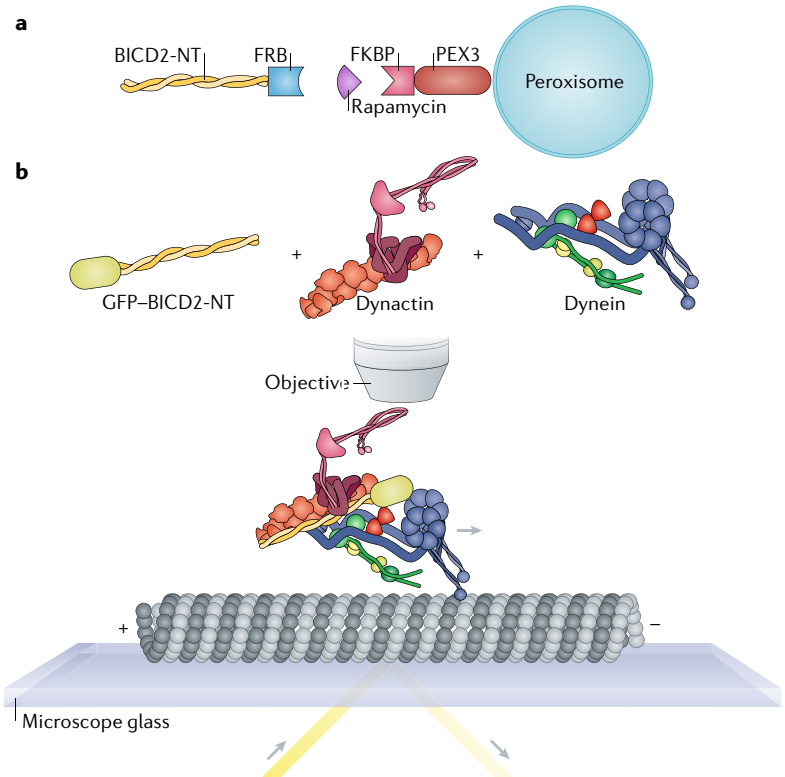
Box 2 | **Methods to study dynein function and activation**

Components of the dynein machinery have been depleted using RNAi and CRISPR methods, and genetic studies have revealed the functions of many components. Several other widely used methods have been important for determining if dynein or dynactin are involved in a process of interest. Overexpression of dynactin components, such as p50 (REF.[221]) (FIG. 1c) or one of the coiled-coil segments (CC1) of p150 (REF.[222]) (FIG. 1c), results in the disruption of many dynein–dynactin-dependent processes. The exact mechanism of disruption is not completely clear but probably involves disruption of the dynein–dynactin interaction[223]. Antibodies raised against the dynein intermediate chains (DICs) (FIG. 1a) have also been extensively used to block dynein function in cell-free systems or systems amenable to antibody injection[224].

A number of methods have been used to provide evidence that a dynein adaptor is an activating adaptor. All activating adaptors co-immunoprecipitate with dynein and dynactin, providing an initial suggestion that a candidate adaptor is an activating adaptor[98]. Cell-based relocation assays have also been used to provide evidence for dynein–dynactin activation[225,226]. In these experiments, a candidate activator is targeted to a largely non-motile organelle, such as the peroxisome. Any increase in minus-end-directed peroxisome motility provides indirect evidence that a candidate adaptor could be an activating adaptor. For example, the amino terminus (NT) of protein bicaudal D homologue 2 (BICD2) can be fused to the rapamycin-binding domain FRB. FKBP, which also binds rapamycin, is fused to an organelle-targeting protein, such as the peroxisomal protein peroxisomal biogenesis factor 3 (PEX3). In the presence of rapamycin, BICD2 enhances peroxisome motility[226] (see figure part **a**).

In vitro motility experiments can also provide indirect evidence for activating adaptors. For example, candidate activators can be used to immunoprecipitate dynein and dynactin from cell lysates or tissue extracts, and the motile properties of the immunoprecipitated complex can then be assessed using single-molecule motility assays[14,98]. A variation of this method is to visualize dynein or dynactin directly in lysates without prior immunoprecipitation[227].

The gold standard for determining if a candidate adaptor is a dynein–dynactin activator is to reconstitute motility from purified components, as has been done for BICD2, BICD family-like cargo adapter 1 (BICDL1; also known as BICDR1), protein Hook homologue 3 (HOOK3), ninein (NIN) and ninein-like (NINL)[14–16,24,98]. In this approach, each protein or protein complex is purified separately, and at least one component is tagged with a fluorescent marker. Motility is then monitored using total internal reflection microscopy. Bond fide activators lead to processive dynein–dynactin motility (see figure part **b**).



overexpression of p50 (REF.[51]), and dynein co-localizes with markers of the ERGIC compartment[52].

It is not yet clear how dynein associates with either the ER or the ERGIC. These interactions could either be direct or mediated by attaching to another vesicle that then interacts with dynein. The latter process, called 'hitchhiking', has been observed in filamentous fungi[53]. In *Ustilago maydis*, ER vesicles co-migrate with early endosomes[54], a cargo that requires dynein for movement in this organism[55]. It remains to be seen if ER hitchhiking is conserved in mammalian cells.

Golgi apparatus. The Golgi apparatus is made up of stacks of membranes typically positioned near the nucleus. However, in some organisms and cell types, the Golgi stacks are dispersed throughout the cytoplasm. In Golgi stacks, secreted proteins move from the nuclear proximal *cis*-Golgi compartment through the stack to the *trans*-Golgi network (TGN). From the TGN, components are transported in Golgi-derived vesicles to the plasma membrane or to other organelles, such as endosomes and lysosomes.

Dynein has a key role in Golgi positioning. The Golgi is dispersed by deletion of dynein in mouse cells[7], injection of anti-DIC antibodies or overexpression of p50 (REF.[56]) (BOX 2). The BICD activating adaptors (BICD1 and BICD2), which are involved in Golgi vesicle movement (see below), are not involved in this process, as overexpression of a C-terminal fragment of BICD2 displaces endogenous BICD2 but has no effect on Golgi morphology[57,58]. A candidate adaptor for dynein-based Golgi positioning is golgin subfamily A member 3 (GOLGA3) as its knockdown leads to Golgi dispersal, and it co-immunoprecipitates with both dynein and dynactin. When GOLGA3 is ectopically recruited to non-Golgi membranes, it drives their movement towards the centre of the cell (relocation assay, BOX 2)[59], which would be consistent with it acting as an activating adaptor for dynein–dynactin. While GOLGA3 is rich in coiled coils, it lacks any obvious DLIC-interacting motif, and the region of the protein used in the relocation assay is too short to activate dynein in a BICD2-like manner. Therefore, it is an open question whether GOLGA3 is a true activating adaptor.

Dynein has been directly linked to the movement of Golgi-derived vesicles via activating adaptors of the BICD family. In addition to bridging the dynein–dynactin interaction, BICD2 binds the RAB6 GTPase and localizes to cytoplasmic vesicles and the TGN[57,58] (FIG. 3a). RAB6-marked vesicles have been implicated in intra-Golgi, endosome-to-Golgi, Golgi-to-ER and Golgi-derived exocytic vesicle trafficking[48]. Overexpression of a C-terminal fragment of BICD2 displaces endogenous BICD2 from RAB6 vesicles and causes them to accumulate at the periphery of the cell[57,58]. This is presumably due to kinesin-driven transport dominating when dynein–dynactin is removed from the RAB6 vesicles[60].

BICDL1 is another Golgi-associated activating adaptor, which also binds RAB6 and is predominantly associated with exocytic Golgi-derived vesicles[61]. Unlike BICD1 and BICD2, BICDL1 is expressed predominantly early in embryonic development in neural and kidney tissue and regulates neurite outgrowth[61].

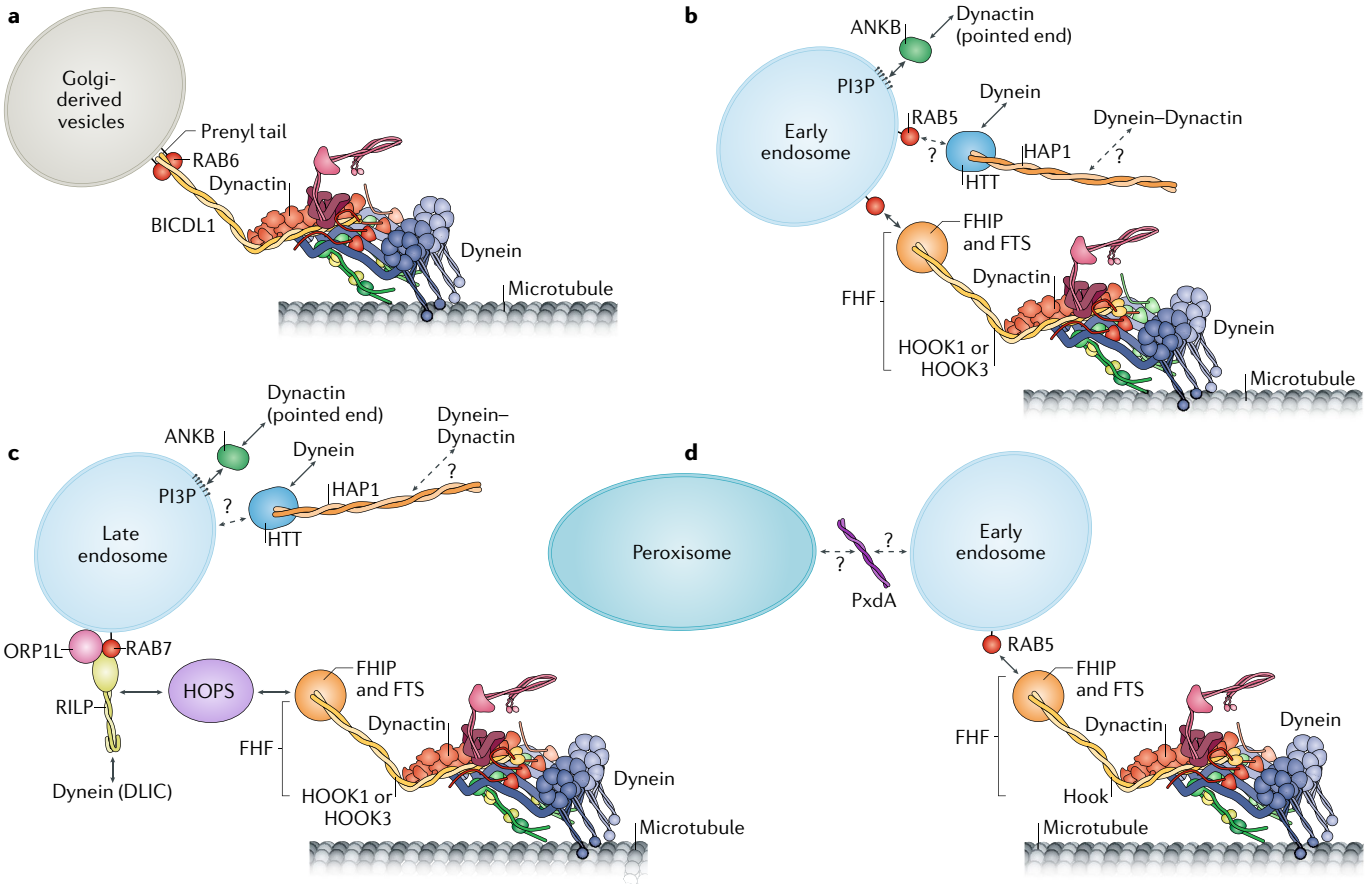


Fig. 3 | **Mechanisms linking dynein and dynactin to membrane cargoes. a** | Dynein–dynactin associates with Golgi-derived vesicles using the activating adaptors protein bicaudal D homologue 2 (BICD2) or BICD family-like cargo adapter 1 (BICDL1; also known as BICDR1), depending on the cell type. Both BICD2 and BICDL1 bind, via their C-terminal coiled coils, to a dimer of the small GTPase RAS-related protein RAB6. RAB6 binds to Golgi-derived membranes via its prenyl tails, as do other RABs[48]. **b** | Early endosomes recruit dynein–dynactin via the activating adaptors protein Hook homologue 1 (HOOK1) or HOOK3. These adaptors bind AKT-interacting protein (FTS; also known as AKTIP), and FTS and hook-interacting protein (FHIP) to form the FHF complex. FHIP is reported to bind directly to the early endosome marker RAB5. RAB5 also binds huntingtin (HTT), which is linked to huntingtin-interacting protein 1 (HAP1), another potential activating adaptor. The phosphatidylinositol 3-phosphate (PI3P)-binding protein ankyrin 2 (ANKB) binds the pointed end of dynactin and is also important for early endosome transport. How these and other dynein–dynactin adaptors work together is unknown. **c** | Late endosomes are marked with RAB7, which binds RAB-interacting lysosomal protein (RILP) and the cholesterol sensor oxysterol-binding protein-related protein 1 (ORP1L). RILP binds the dynein light intermediate chain (DLIC). RILP also binds the HOPS complex, which interacts with the FHF complex, raising the possibility that Hook proteins also link dynein–dynactin to late endosomes. As with early endosomes, other potential dynein–dynactin links have been reported for the movement of late endosomes. **d** | In filamentous fungi, some cargoes (peroxisomes, endoplasmic reticulum-membrane-derived vesicles, lipid droplets and ribonucleoproteins) associate with the dynein transport machinery indirectly by hitchhiking on early endosomes, which can directly recruit the transport machinery via the Hook-containing FHF complex (see part **b**). PxdA is a putative tether that links peroxisomes to early endosomes, although how it may interact with both peroxisomes and early endosomes is unknown.

A related isoform, BICDL2, binds to RAB13 (REF.[61]) and is also associated with post-Golgi trafficking[62]. The activating adaptor NINL and the candidate activating adaptor HOOK2 (TABLE 1) have both been implicated in trafficking RAB8-marked Golgi-derived vesicles that are destined for the base of the primary cilium[63,64].

Endolysosomal system. Endocytosis is the process of internalizing portions of the plasma membrane, which can contain receptors and their ligands. Internalized membranes are sorted to various destinations, including recycling endosomes, late endosomes, multivesicular bodies (MVBs), the TGN and the lysosome. Each compartment is marked by one or more RAB GTPases.

Dynein is required for trafficking throughout the endolysosomal system. For example, knockout of dynein in mouse cells disperses lysosomes and endosomes[7]. Overexpression of p50 disrupts early and late endosome and lysosome distribution[56], inhibits trafficking of signalling receptors from the cell surface towards the centre of the cell[65] and blocks transport of endosomes along nerve axons[66].

Early endosomes are marked with the RAB5 GTPase. Activating adaptors of the Hook family have been

Total internal reflection microscopy
A microscopy technique that results in illumination of only a region approximately 100 nm from the coverslip surface, allowing high signal-to-noise ratios to be achieved, which makes it feasible to image and track single molecules.

Recycling endosomes
Endocytic vesicles characterized by the presence of the RAS-related protein RAB11 that direct the anterograde trafficking of materials to the cell surface.

Late endosomes
Pre-lysosomal endocytic vesicles with lower internal pH relative to early endosomes and characterized by the presence of the protein RAS-related protein RAB7a (RAB7).

Multivesicular bodies
(MVBs). Late endosomes that contain multiple internalized vesicles.

HOPS complex
A multisubunit membrane-tethering complex that participates in organelle fusion events within the endolysosomal system in concert with RAB proteins.

implicated in linking these vesicles to dynein. Hook was first shown to have a role in endocytic trafficking in *D. melanogaster*[67]. Experiments in filamentous fungi subsequently linked Hook to dynein, showing that a Hook homologue was required to link Rab5-marked early endosomes to the dynein machinery[68,69]. Mammals have three Hook-related proteins (HOOK1, HOOK2 and HOOK3). HOOK1 and HOOK3 have been directly linked to dynein-driven movement of early endosomes in axons[70].

Hook proteins are part of a complex called FHF, named after its components FTS (encoded by *AKTIP*), a Hook-related protein, and FTS and hook-interacting protein (FHIP, encoded by *FAM160A2*)[68,71,72]. The GTP-bound form of RAB5 interacts with the FHF complex in both *D. melanogaster*[73] and human cell[70] extracts. In *Aspergillus nidulans*, FhipA (FHIP) can bind to early endosomes in the absence of the other two components[72], and two-hybrid interaction studies with human proteins suggest that FHIP binds directly to the GTP-bound form of RAB5 (REF.[70]). This suggests the FHF complex binds directly to RAB5 on early endosomes via the FHIP component, and the Hook protein recruits the dynein–dynactin complex (FIG. 3b).

Another potential connection between dynein and early endosomes is via huntingtin (HTT) and HAP1 (BOX 1; FIG. 1d). HTT binds to the GTP-bound form of RAB5 (REF.[73]) and has been linked to the movement of signalling endosomes (see also below)[74] and other membranous cargoes. In addition, HTT binds dynein[75], and HAP1 binds dynactin[76]. HAP1 is a candidate activating adaptor based on its homology to other adaptors (TABLE 1; FIG. 1d). An intriguing question is whether both HTT–HAP1 and the FHF complex are found on the same early endosomes or whether they provide alternate routes to recruit dynein–dynactin (FIG. 3b).

Late endosomes are marked with the GTPase RAB7. RAB7 forms a complex with the cholesterol sensor oxysterol-binding protein-related protein 1 (ORP1L) and RILP[77] (BOX 1). RILP binds to GTP-bound RAB7 (REF.[78]), directly binds the DLIC[23,35] and is required to recruit dynein and dynactin to late endosomes and lysosomes[34] (FIG. 3c). Interestingly, the ORP1L–RAB7–RILP complex also interacts with the HOPS complex[71], which is implicated in late endosome trafficking[79]. The HOPS complex binds to the Hook-containing FHF complex[80,81], raising the possibility that late endosomes also engage Hook proteins to link them to dynein[82] (FIG. 3c). If this is confirmed, it will suggest that, as with early endosomes, there are multiple ways in which dynein can be recruited to late endosomes. Late endosomes mature into lysosomes, which are found in a perinuclear region in some cell types. This positioning has also been linked to dynein–dynactin[56], and there is evidence that lysosome motility and positioning are mediated by the same factors that are used by late endosomes[83].

In addition to the adaptors described above, a number of other proteins have been implicated in the association of dynein with the endolysosomal system, including JIP3 (REFS[33,84]), ankyrin B[85], snapin[86] and spectrin[87] (FIG. 3b,c; BOX 1). These proteins all lack long coiled coils, suggesting they are not activating adaptors. This raises the question of whether these proteins act together with an activating adaptor or can provide an alternate means of activating dynein–dynactin.

A subset of endosomes contains receptors that signal in the cytoplasm after internalization. These endosomes, called signalling endosomes, have been well studied in neurons, where growth factors bind to receptors at the nerve synapse, undergo endocytosis and are transported back along the axon to the cell body by dynein–dynactin. For example, β-nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) bind to the high-affinity nerve growth factor receptor (TRKA) and BDNF/NT-3 growth factors receptor (TRKB), respectively[88]. These NGF–TRKA and BDNF–TRKB complexes co-localize with both RAB5-marked early endosomes[89] and RAB7-marked late endosomes in axons[90], suggesting that signalling endosomes use the same adaptors as early and late endosomes.

Many cells also have an endosomal compartment near the MTOC that is a nexus for receptor recycling to the plasma membrane and is distinct from early endosomes. These recycling endosomes are marked by RAB11 (REF.[91]). RAB11FIP3 is a RAB11-interacting protein that is required to maintain the structure of recycling endosomes[92] and activates dynein–dynactin motility in vitro[14] (FIG. 1d; TABLE 1). RAB11FIP3 is also required to deliver membranes to cilia[93] and the cytokinetic furrow[94]. As with endosomes, other adaptors may also recruit dynein–dynactin to recycling endosomes. For example, JIP3 has been shown to have a role in the movement of recycling endosomes during cytokinesis[95].

Melanosomes. Melanosomes are pigment-containing organelles related to lysosomes. In many vertebrates, their movements are regulated to control skin colour changes. In pigmented cells of *Xenopus laevis*, antibodies against the DLIC block melanosome movement towards the cell centre[96]. In zebrafish lacking the protein Ninl, melanosome transport is severely impaired. NINL interacts with components of the dynein and dynactin complex as shown via mass spectrometry experiments[97,98], and human NINL is an activating adaptor[98], suggesting that NINL is the activating adaptor for melanosome motility.

Autophagosomes. Autophagosomes are double-membrane vesicles formed after engulfment of organelles and proteins destined for destruction by autophagy. They are marked with the ubiquitin-related protein LC3. In HeLa cells, autophagosomes move to the cell centre and cluster with lysosomes[99]. In neurons, autophagosomes can form at the axon tip[100] and then fuse with lysosome-associated membrane glycoprotein 1 (LAMP1)-marked or RAB7-marked late endosomes to initiate transport towards the cell body[100–102]. Autophagosomes can also form in the soma[103].

A role for dynein in autophagy came from the observation that overexpression of p50 or p150 CC1 (BOX 2) impairs autophagic clearance[104]. Furthermore, injection of an anti-DIC antibody inhibits clustering of LC3 autophagosomes in HeLa cells[99], and p150 CC1 overexpression inhibits their movement in neurons[100]. Because moving autophagosomes can fuse with late endosomes,

Pronuclei
The distinct egg and sperm nuclei that are present within a single cell at the onset of fertilization before the fusion of their genetic material.

Interkinetic nuclear migration
The cell cycle-dependent movement of nuclei observed in neural progenitor cells.

Syncytial blastoderm
Tissue that builds early *Drosophila melanogaster* embryo and that is characterized by multiple nuclei residing in a shared cytoplasm, which is the result of multiple nuclear divisions in the absence of cytokinesis.

a Hook protein may be the activating adaptor for dynein-mediated autophagosome transport. There is also evidence that HTT and the candidate activating adaptor HAP1 are important for autophagosome motility[105]. JIP1 is also necessary for autophagosome motility[105,106]. JIP1 is structurally unrelated to JIP3 and lacks any predicted coiled coil, suggesting it is not an activating adaptor. Intriguingly, autophagosomes have different motile properties compared with endosomes and lysosomes[107]. It is not yet clear what accounts for these differences.

Mitochondria. Mitochondria move bidirectionally along microtubules and pause frequently. Their motility can respond to, for example, changes in cell signalling and axon growth, which can lead to their accumulation in areas with high metabolic requirements[108]. Mutations in *D. melanogaster* dynein impair movement of mitochondria in axons, suggesting a direct role of dynein in retrograde movement (from the periphery to the cell body) of mitochondria[109]. Both dynein and kinesin associate with mitochondria via the protein Milton (TRAK1 and TRAK2 in humans), which binds to the mitochondrial outer membrane protein Miro (RHOT1 and RHOT2 in humans)[110]. TRAK proteins co-precipitate dynein and dynactin from brain extracts[111], suggesting they interact with the dynein motor. TRAK1 and TRAK2 contain a region with similarity to BICDL1 and HAP1[60], raising the possibility that the TRAK proteins are activating adaptors of dynein–dynactin for mitochondrial motility (BOX 1; FIG. 1d).

Peroxisomes. Peroxisomes perform a variety of metabolic functions, including the breakdown of fatty acids and metabolism of hydrogen peroxide. Peroxisomes are relatively evenly distributed in cells, and only 10–15% of them are mobile in many cell types[112]. Overexpression of p50 disrupts peroxisome motility in mammalian cells[113]. The candidate activating adaptor TRAK2 has been implicated in peroxisome motility, along with a splice variant of the TRAK-binding protein mitochondrial Rho GTPase 1 (RHOT1; also known as Milton in *D. melanogaster*)[114].

In filamentous fungi, where peroxisomes move in a kinesin-dependent and dynein-dependent manner[115], they do so by hitchhiking on moving early endosomes[54,116]. Peroxisomes co-migrate with RabA (Rab5)-marked early endosomes and require endosome motility for movement. In *A. nidulans*, PxdA, a long coiled-coil-containing protein is required for hitchhiking and may act to tether the two organelles[116] (FIG. 3d). It is not clear if the hitchhiking mechanism is used for peroxisome motility outside of filamentous fungi.

Lipid droplets. Lipid droplets serve as lipid storage compartments and also provide a source of membrane lipid precursors[117]. Like peroxisomes, they show both diffusive and microtubule-dependent movements[118]. Genetic studies in *D. melanogaster* and filamentous fungi have implicated kinesin and dynein in the bidirectional motility of lipid droplets[54,119,120]. In *D. melanogaster*, BicD, which is related to the mammalian BICD proteins, has been shown to have a role in lipid droplet movement[121]. However, it is unclear if this role is direct, and no other adaptor proteins have been identified for lipid droplets to date. In the filamentous fungus *U. maydis*, lipid droplets hitchhike on early endosomes, similar to ER vesicles and peroxisomes[54].

Nuclei. Dynein-dependent movement and positioning of nuclei occurs in many organisms ranging from yeast to humans. In mitosis, dynein positions the spindle, which ultimately leads to nuclear positioning[122,123]. In interphase cells, dynein also has direct roles in nuclear movement or positioning. Early demonstrations of this included the discoveries that filamentous fungi with mutations in dynein or dynactin subunits had nuclear positioining defects[124,125] and *Caenorhabditis elegans* embryos depleted of dynein or dynactin had defects in pronuclei movement[126]. In vertebrate cells, dynein is responsible for the movement of nuclei during cell migration[127,128], interkinetic nuclear migration in neural progenitor cells[129], and distributing and positioning nuclei in developing muscle cells (myoblasts)[130,131].

These different nuclear movements can be driven by dynein that is directly connected to the nucleus or by cortically anchored dynein pulling on microtubules emanating from MTOCs. A number of proteins have been identified as being important for linking dynein to the cell cortex or nuclear membrane. In mitosis, NUMA and the G-protein-signalling modulator 2 (GPSM2; also known as LGN) complex anchor dynein to the cortex[132]. In interphase, dynein is anchored to the nucleus by BICD2 via the nucleoporin E3 SUMO-protein ligase RANBP2 (REFS[133,134]). The nuclear pore complex protein NUP133 and Nudel proteins also have a role in localizing dynein to the nucleus[133,135]. In some cell types, dynein may be recruited by different mechanisms. For example, partitioning defective 6 homologue β (PAR6β) is required for dynactin localization to the nuclear envelope in myotubes, and its depletion reduces the movement of recently fused myoblast nuclei within myotubes[130]. In *C. elegans*, a protein related to the Hook family of activating adaptors, ZYG-12, is important for attaching dynein to the nuclear membrane[136].

RNA cargoes

Subcellular localization of mRNAs is a mechanism to locally control gene expression in many organisms[137]. The role of dynein in RNA localization was discovered in *D. melanogaster*[138], where mutations in the DHC or injection of antibodies against the DHC or p50 all lead to defects in mRNA localization in the fly syncytial blastoderm[139]. In mammalian cells, there is no direct evidence for the role of dynein in RNA localization, although the DIC co-immunoprecipitates with the RNA-binding proteins Staufen[140] and La[141].

mRNAs are transported in complex with proteins as RNPs. In *D. melanogaster*, the localization of many RNAs requires BicD, and the formation of transport competent mRNP complexes requires the RNA-binding protein Egalitarian (Egl). Egl binds directly to RNA and the C-terminal cargo-binding domain of BicD, thus linking RNA to the dynein transport machinery[142]. The dynein–dynactin–BicD–Egl complex has an essential

Nurse cells
A group of 15 polyploid *Drosophila melanogaster* ovarian cells that share a cytoplasm with each other and the developing oocyte and function to support the development of the oocyte by providing nutrients and biomolecules (mRNAs and proteins) through intercellular connections called ring canals.

Dendritic branching
The process by which a dendrite, the portion of neuron that receives signals from other cells, forms the cellular projections it contributes to synapses.

Polysomes
The complex formed by two or more ribosomes simultaneously engaged in translation along the length of a single messenger RNA.

14-3-3 protein
A conserved family of adaptor proteins that interact with diverse proteins and regulate their function through, for example, altered localization, activity or stability.

Importins
Proteins that recognize and deliver proteins with nuclear localization signals into the nucleus through nuclear pores.

γ-tubulin
Tubulin family member that, as a component of γ-tubulin ring complexes, templates nascent microtubules.

role during oogenesis in flies by controlling the localization of *oskar* and *bicoid* mRNAs, which are involved in anterior–posterior axis determination of the embryo[143]. These mRNAs, packaged as mRNPs, are transported from nurse cells into the oocyte along polarized microtubule arrays, requiring dynein, dynactin, BicD and Egl for movement[144,145]. Once inside the oocyte, *bicoid* continues to require dynein to maintain its localization[146]. BicD also contributes to RNA localization in fly neurons, where it interacts with the RNA-binding factor nuclear fragile X mental retardation-interacting protein 2 (NUFIP2; also known as FMRP)[147]. BicD and FMRP interact and move together bidirectionally in fly neurons, and both proteins are required for normal levels of dendritic branching[147].

In filamentous fungi, RNAs are distributed by hitchhiking on early endosomes[148]. For example, in *U. maydis*, mRNA-containing polysomes are distributed throughout the cytoplasm by their association with dynein and kinesin-driven early endosomes[149,150]. It remains to be explored if hitchhiking is used by higher eukaryotes as a means to distribute or localize mRNA transcripts.

Protein cargoes

Aggresomes and misfolded proteins. Misfolded proteins are processed either by chaperones, which refold them, or the ubiquitin-proteasome system, which removes them by proteolysis. When these pathways are overwhelmed, cells temporarily sequester these toxic protein species. The misfolded proteins are transported to juxtanuclear structures known as aggresomes that can be eventually processed by autophagy[151]. Overexpression of p50 (BOX 1) prevents the formation of aggresomes, implicating dynein–dynactin in their formation[152,153]. Three pathways have been reported to recognize misfolded proteins and connect them to dynein–dynactin. The first depends on histone deacetylase 6 (HDAC6), which links polyubiquitylated proteins to dynein–dynactin[154]. The precise protein–protein interactions underlying the connection of HDAC6 to dynein–dynactin are not known. A second pathway uses sequestosome 1 (SQSTM1) to link polyubiquitylated proteins to dynein through an interaction with the DIC[154]. HDAC6 and SQSTM1 interact with each other[155], although depletion of HDAC6 increases the amount of SQSTM1 that interacts with dynein, suggesting that HDAC6-dependent and SQTSM1-dependent pathways act competitively[156]. A third pathway uses the heat shock protein 70 (HSP70) co-chaperone BAG family molecular chaperone regulator 3 (BAG3) to couple misfolded proteins to the dynein–dynactin complex. This pathway does not require ubiquitylation to target proteins to the aggresome[157]. The BAG3–dynein interaction is bridged by the 14-3-3 protein, which interacts with BAG3 and the DIC[158]. No known activating adaptor has so far been implicated in aggresome formation.

Transcription factors. Although small proteins can move quickly by diffusion, there is evidence that some are transported by dynein. For example, transcription factors are translated locally in axons in response to nerve injury and then transported in a retrograde direction along microtubules. This relocalizes them to the nucleus where they activate a transcriptional response[159]. The transcription factor signal transducers and activators of transcription 3 (STAT3) immunoprecipitates with dynein, and disruption of microtubules prevents its accumulation in the nucleus in response to axon damage[160]. The connection between dynein and STAT3 may involve importins, as a peptide that blocks the STAT3 interaction with importin reduces the ability of STAT3 to immunoprecipitate with dynein[160,161].

Intermediate filaments and microtubules. Intermediate filaments are cytoskeletal components that contribute to cell shape, motility and organelle positioning[162]. Dynein participates in the subcellular distribution of vimentin intermediate filaments. Vimentin moves along microtubules[163], and overexpression of p50 (BOX 1) redistributes vimentin from the perinuclear region to the cell periphery[164]. In addition, vimentin co-immunoprecipitates with dynactin[165]. Depletion of the DHC also impairs the retrograde motility of intermediate filaments present in neurons, known as neurofilaments[166]. This might reflect a direct interaction of dynein with some neurofilaments, as neurofilament M interacts with the DIC[167].

It has also been shown that dynein can contribute to the movement of microtubules. Cell body-originating microtubules are transported into developing axons in rat neuron cultures via a process that is blocked by overexpression of p50 (REF.[168]) (BOX 1). Because these dynein-dependent microtubule movements are anterograde (in the opposite direction from normal dynein transport in the axon), it suggests that the microtubules move by sliding over dynein anchored to the cortex[166]. Cortically anchored dynein also drives the formation of a uniformly polarized microtubule network in *D. melanogaster* neurons[169].

Centrosomal components. Centrosomes are the sites of microtubule nucleation and typically anchor microtubule minus ends[170]. Dynein has a role in transporting proteins to the centrosome. For example, overexpression of the CC1 domain of the dynactin subunit p150 (BOX 2) disrupts the localization of CDK5 regulatory subunit-associated protein 2 (CDK5RAP2; also known as CEP215) to the centrosome[171]. CDK5RAP2 is required for γ-tubulin localization and microtubule polymerization[172]. CDK5RAP2 contains a long region of a predicted coiled coil and co-immunoprecipitates with the DIC, although further work will be required to determine if it is an activating adaptor for dynein cargoes destined for the centrosome. There is evidence that the known or candidate activating adaptors NIN, NINL and HOOK2 are enriched at the centrosome[173–175]. NIN and NINL have a role in nucleating microtubules, suggesting that they transport factors required for this process to centrosomes.

Centriolar satellites are smaller motile structures that contribute material to centrosomes and are important for ciliogenesis[176]. Dynein interacts with components of satellites[177], and overexpression of p50 (BOX 1) disperses them[178]. In neuroblastoma cells, the activating adaptor HOOK3 localizes to and is important for the function of centriolar satellites, raising the possibility that HOOK3

Table 2 | **Viruses interacting with dynein during their life cycle**

Virus	Presence of the viral envelope	Replication site	Role of dynein in viral life cycle	Evidence for dynein involvement
Class 1 (dsDNA)				
HSV	Env	Nuc	Transport (direct)	HSV movement requires microtubules[236]; p50 blocks movement[236]
Pseudorabies virus	Env	Nuc	Transport (direct)	GFP-tagged capsids move retrograde in axons[237]; pUL36 on mitochondria relocates them to perinuclear regions[196]
Adenovirus	Non-env	Nuc	Transport (direct)	Viruses do not co-localize with endosomes by electron microscopy; p50 blocks perinuclear accumulation[197]
Polyomavirus	Non-env	Nuc	Transport (vesicles?)	p50 blocks virus at cell periphery[238]
Bovine papilloma viruses	Non-env	Nuc	Transport (vesicles)	Transport in endosomes; viruses remain at the periphery with nocodazole treatment[239]
Vaccinia virus (pox virus)	Env	Cyt	Assembly (direct)	p50 blocks perinuclear accumulation of newly assembled virus particles[187]
African swine fever virus	Env	Cyt	Assembly (?)	p50 blocks viral replication at perinuclear regions[185]
Class II (ssDNA)				
Adeno-associated virus (parvovirus)	Non-env (replication defective)	Nuc	Transport (vesicles)	Axonal transport of labelled viruses in RAB7 vesicles[181]
Circovirus	Non-env	Nuc	Transport (vesicles)	Nocodozaole disrupts perinuclear accumulation[180]
Canine parvovirus	Non-env	Nuc	Transport (direct?)	Anti-dynein antibody reduces perinuclear accumulation[199]
Class III (dsRNA)				
Reoviruses (for example, rotavirus)	Non-env	Cyt	Transport (vesicles)	Viral particles in endosomes accumulate in perinuclear regions[186]
Class IV (+ssRNA)				
Dengue virus (flavivirus)	Env	Cyt	Transport (vesicles) and Assembly (vesicles)	Viruses co-localize with endosomes and accumulate in perinuclear regions[189]
Hepatitis C (flavivirus)	Env	Cyt	Cell organization	HCV clustering of lipid droplets in perinuclear regions[191] requires viral protein NS5A and dynein[240]
Class V (-ssRNA)				
Influenza virus	Env	Cyt	Transport (vesicles)	Anti-dynein antibody inhibits rapid endosome transport to perinuclear regions[183]
Rabies virus	Env	Cyt	Transport (vesicles)	Long distance tracking of labelled virus[190]; rabies envelope G protein can confer retrograde transport[200]
Hantaan virus	Env	Cyt	Transport (direct)	p50 blocks perinuclear accumulation of N proteins expressed recombinantly or upon viral infection[188]
Class VI (ssRNA-RT)				
Human foamy virus	Env	Cyt and nuc	Transport (vesicles)	p50 blocks perinuclear accumulation of viruses or isolated Gag proteins[184]
Mason-Pfizer monkey virus	Env	Cyt and nuc	Transport (direct)	p50 blocks perinuclear accumulation of Gag polyproteins[241]
Class VII (dsDNA-RT)				
Hepatitis B	Env	Cyt and nuc	Cell organization	Triggers dynein clustering of mitochondria in perinuclear regions[192]

Viruses are listed by class: classification based on nucleic acid (DNA or RNA), strandedness (ss, single-stranded; or ds, double-stranded), sense (+ strand or – strand) and whether they use a reverse transcriptase (RT). Dynein is involved in different aspects of the viral life cycle, irrespective of whether the viruses are enveloped (Env) or non-enveloped (Non-env), or whether they replicate in the nucleus (Nuc) or cytoplasm (Cyt). Roles of dynein can include transport of viruses to the perinuclear region during infection (via a direct connection or by transporting them in endosomes). Dynein can also transport components during assembly of new viruses or can have a role reorganizing the cell in response to viral infection. Evidence for the role of dynein in viral infection is accumulation near the nucleus (perinuclear). Further support comes from showing that the viral components are dispersed or fail to accumulate when nocodazole is used to depolymerize microtubules, p50 is overexpressed (p50 block) or an anti-dynein antibody is injected. Direct visualization of viruses moving in the retrograde direction in axons also indicates a role for dynein. HCV, hepatitis C virus; HSV, herpes simplex virus; NS5A, genome polyprotein; N, nucleocapsid; RAB, RAS-related protein.

activates dynein–dynactin to drive the movement of satellite components towards the MTOC[179].

Finally, dynein also has a role in tethering centrosomes to the nucleus. Depletion of BICD2 or RANBP2, which are located in the nucleus, severs the tight association of centrosomes and the nucleus[134].

Viruses

There are seven classes of viruses, and members from every class use dynein for some aspect of their life cycle (TABLE 2). The most common roles are related to viral replication. For example, DNA and RNA viruses that replicate in the nucleus use dynein motility to reach it[180–183]. Some retroviruses, which reverse transcribe their RNA genomes into DNA in the cytoplasm, use dynein to deliver the DNA to the nucleus for integration into the nuclear genome[184]. In addition, some DNA and RNA viruses that replicate in the cytoplasm in 'perinuclear factories' also require dynein to accumulate at these sites[185–189].

In addition to using dynein to reach their site of replication, some viruses use dynein at other stages of their life cycles. Rabies virus uses dynein for motility along neural axons, which allows it to spread from the site of infection to other parts of the nervous system[190]. Dengue virus may use dynein during the assembly of new viral particles[189]. Hepatitis C virus triggers dynein-dependent clustering of lipid droplets at the MTOC, which are then incorporated into newly formed viral particles[191]. Hepatitis B virus uses dynein to cluster mitochondria close to the nucleus, perhaps to provide energy for its replication[192]. Finally, there is evidence that influenza virus requires dynein to disassemble the viral capsid to release its RNA to the cytoplasm. HDAC6 is also required for this release step, suggesting that HDAC6 serves as a dynein adaptor in this context[193].

The connections between viruses and dynein during viral particle transport can either be direct or indirect. Examples of viruses that use direct interactions include herpes simplex virus and pseudorabies virus[194]. In these cases, the viral inner tegument proteins (which are attached to the capsid) are required for dynein binding[195]. The prime candidate for interaction with dynein is pUL36, which is a potential activating adaptor of dynein, as it is active in a relocation assay[196] (BOX 2). Adenoviral particles may also travel by direct interaction with dynein after infection and may use this transport to reach the nucleus[197]. The adenovirus capsid protein, hexon, immunoprecipitates with dynein and interacts with both the DLIC and DIC[198]. The interaction with dynein is stimulated by phosphorylation of the RAS-like domain of DLIC[35], suggesting a direct interaction with dynein. However, the role of dynactin in adenoviral intracellular transport is less clear, as dynactin did not immunoprecipitate with hexon[198], but p50 overexpression (BOX 1) blocked nuclear accumulation of the adenovirus[197]. There is also evidence that other viruses interact directly with dynein–dynactin[184,188,199].

Examples of indirect dynein-based transport involve viruses that move intracellularly within endosomes. Adeno-associated viral particles, for example, which move along neural axons, co-localize with RAB7 vesicles[181]. Rabies viral particles also move within vesicles and, in this form, are transported along neuronal axons[190]. Their dynein-directed movement depends on the envelope glycoprotein, as it was shown that incorporating this protein into retroviral capsids allows retrograde transport of retroviral particles along axons[200]. How the viral glycoprotein regulates dynein is a mystery, as it is localized within the moving endosome rather than on its cytoplasmic face.

Conclusions and perspectives

While a vast number of dynein cargoes have been described, there are likely to be many more. For example, recent proteomic experiments have identified a number of putative new cargoes linked to dynein by the BICD1, BICD2, HOOK1, HOOK3, NIN or NINL activating adaptors[98]. Here, we have focused largely on cargoes that dynein translocates along microtubules, but dynein can also function anchored at the cell cortex, where it can capture the plus ends of dynamic microtubules[201]. New dynein cargoes may include specific cortical sites that anchor dynein, as suggested by studies that find dynein on the cortex at adherens junctions[202] and focal adhesions[203], and the numerous cortically localized proteins found in the dynein interactome[98].

This large number of dynein cargoes raises many questions related to how dynein achieves cargo specificity. What molecular interactions mediate binding of dynein to new cargoes? Do some cargoes hitchhike rather than recruit dynein directly[53]?

In this Review, we have emphasized the role of activating adaptors in dynein–dynactin motility. A question for the future will be to determine if all dynein cargoes, including viruses, require an activating adaptor. Reconstitution experiments will be required to verify if all current candidate activating adaptors (that is, BICD1, HOOK2, CCDC88A, CCDC88B, CCDC88C, TRAK1, TRAK2, NUMA and HAP1) are indeed able to activate dynein–dynactin motility. If there are fewer activating adaptors than cargoes, how does dynein achieve cargo specificity? Additional proteins that are not activating adaptors are likely required to regulate dynein. For example, as discussed above, the dynein–dynactin interacting proteins, RILP, JIP3 and HTT may be involved in adding cargo specificity to an already activated dynein–dynactin complex.

There may also be mechanisms to activate dynein that do not require activating adaptors. Presumably, these factors will also release dynein from its autoinhibited ϕ-conformation[18]. It is also possible that large clusters of dynein motors could overcome the need for activating adaptors or even for the interaction with dynactin, as dynein groups can processively move beads in vitro in the absence of both dynactin and any activator[204].

Finally, while we have focused on dynein-based movements in this Review, many cargoes move bidirectionally[205]. Some cargoes that move bidirectionally can switch directions rapidly, implying that there is coordination between motors with opposite polarity. What regulates this coordination? Do activating adaptors, some of which can also bind kinesins[61,98,206], have a role in this process?

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Author contributions
S.L.R.-P., A.P.C. and W.B.R. researched data for the article. S.L.R.-P., A.P.C. and R.D.V. discussed the content of the article, S.L.R.-P., A.P.C., W.B.R. and R.D.V. wrote and edited the article.

Competing interests
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