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Review

# Bicaudal D Family of Motor Adaptors: Linking Dynein Motility to Cargo Binding

Casper C. Hoogenraad[1,*] and Anna Akhmanova[1,*]

**Transport of different intracellular cargoes along cytoskeleton filaments is essential for the morphogenesis and function of a broad variety of eukaryotic cells. Intracellular transport is mediated by cytoskeletal motors including myosin, kinesin, and dynein, which are typically linked to various cargoes by adaptor proteins. Recent studies suggest that adaptor proteins can also act as essential transport cofactors, which control motor activity and coordination. Characterization of the evolutionary conserved Bicaudal D (BICD) family of dynein adaptor proteins has provided important insights into the fundamental mechanisms governing cargo trafficking. This review highlights the advances in the current understanding of how BICD adaptors regulate microtubule-based transport and how they contribute to developmental processes and human disease.**

## Trends

Bicaudal D adaptor proteins link dynein motors to specific cargos.

Bicaudal D and related adaptors induce processive motility by stabilizing the interaction of dynein and dynactin.

Bicaudal D and related adaptors can interact with motors of opposite polarity and control their activities.

Conformational transitions in Bicaudal D may coordinate motor recruitment with cargo binding.

Missense mutations in Bicaudal D and other dynein cofactors lead to transport defects and human disease.

## Intracellular Transport and Motor Adaptor Proteins

Intracellular cargo transport is essential for cellular function, and many developmental and neurological diseases directly result from mutations in various components of transport pathways [1–3]. The basic transport machinery is well defined: the cytoskeleton forms a network of 'rails' that are used by molecular motors for the directed delivery of cargo, such as organelles, vesicles, protein complexes, and mRNAs. Long-range transport depends on microtubules, which are relatively long and rigid cytoskeletal filaments that serve as tracks for kinesin and dynein motors [4,5]. Individual motors move unidirectionally along microtubules, with kinesins typically translocating towards the plus end and dyneins to the minus end. Some cargoes move predominantly in one direction; however, many organelles and macromolecular complexes undergo back-and-forth movements because of the alternating activities of kinesin and dynein motors, which can remain stably associated with the cargo even when they are inactive [6–10]. The net transport and the correct cellular distribution of cargoes thus often depend on the balance between the opposing dynein and kinesin activities. The molecular mechanisms underlying motor recruitment, activation, and regulation are still poorly understood.

Various adaptor proteins that link motors to cargo have been implicated in controlling motor coordination and cargo movement (reviewed in [9–12]). Motor adaptors often represent core components of large protein complexes, which include membrane-associated cargo receptors, scaffolding factors, signaling proteins, such as kinases and GTPases, and even glycolytic enzymes (reviewed in [11,13]) (Figure 1A). Well-studied multifunctional adaptor proteins that regulate cargo transport include Milton/TRAK, Jun N-terminal kinase (JNK)-interacting proteins (JIPs), glutamate receptor-interacting protein (GRIP), Rab7-interacting lysosomal protein (RILP), and sorting nexins [14–18] (Figure 1A). Motor–adaptor complexes can integrate signaling cues, such as changes in $Ca^{2+}$ levels, phosphorylation or Rab GTPase activity, to locally control cargo movement (Figure 1B) (reviewed in [10–12]). In this way, adaptor proteins can facilitate organelle-specific responses to the local environment changes.

[1]Cell Biology, Department of Biology, Faculty of Science, Utrecht University, Padualaan 8, Utrecht, CH 3584 The Netherlands

*Correspondence: c.hoogenraad@uu.nl (C.C. Hoogenraad) and a.akhmanova@uu.nl (A. Akhmanova).

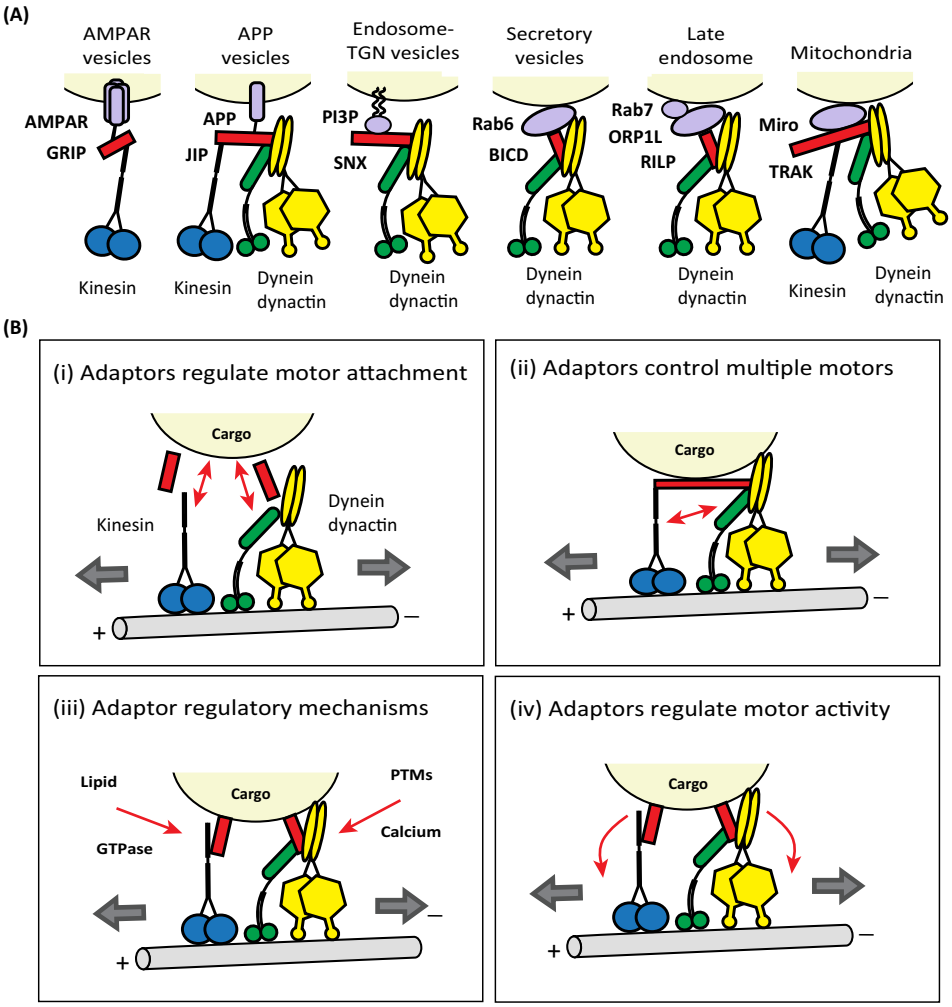


Figure 1. Current Models for Adaptor Proteins Controlling Motor–Cargo Binding. (A) Schematic diagram of various multifunctional adaptor proteins. Adaptor proteins (red) are components of large protein complexes (purple) that link kinesin (blue) and dynein/dynactin motors to various cargoes. Dynein (yellow) and dynactin (green) are indicated (for simplicity) as homodimers with two microtubule-binding domains (circles) and a long tail region (ovals). Most adaptors bind directly to the motor protein but frequently need other factors (such as receptors, membrane proteins, lipids, Rab GTPases, and small G proteins) to interact with the specific cargoes. Examples of multifunctional adaptor proteins: glutamate receptor-interacting protein (GRIP) that binds kinesin-1 and GluA2 containing AMPA receptors; Jun N-terminal kinase (JNK)-interacting proteins (JIPs) that interact with amyloid precursor protein (APP), kinesin-1, and dynein/dynactin; sorting nexin-4 that interacts with phosphoinositides and dynein; Bicaudal D (BICD) that binds to dynein/dynactin and the small GTPase Rab6; Rab7-interacting lysosomal protein (RILP) that associates with dynein, oxysterol-binding protein-related protein 1L (ORP1L); and Milton/TRAK that binds the mitochondrial Rho GTPase Miro, dynein/dynactin, and kinesin-1. (B) Basic functions for motor–cargo adaptor proteins. (i) Adaptors regulate motor attachment: in this model, adaptors interact with one type of motor, either kinesin or dynein–dynactin. If the adaptor recruits kinesin, the cargo will move unidirectionally towards the plus end of the microtubule. If the adaptor binds dynein/dynactin, the cargo will move unidirectionally towards the microtubule minus end. (ii) Adaptors control multiple motors: in this model, adaptors recruit both kinesin and dynein/dynactin motors. The cargo will move bidirectionally along the microtubule, depending on the dominant motor type (tug-of-war mechanism) or the signaling pathways that control motor activity (regulatory mechanism). (iii) Adaptor regulatory mechanisms: adaptor complexes can integrate signaling cues, such as changes in $Ca^{2+}$ levels, phosphorylation, or other post-translational modifications (PTMs), Rab GTPase activity, or organelle-specific phosphoinositide regulation, to control motor–cargo binding and cargo motility. (iv) Adaptors regulate motor activity: in this model, adaptors can act as essential cofactors for motor activation. Bicaudal D (BICD) family proteins have recently been shown to stimulate processive dynein motility by promoting the dynein–dynactin interaction.

Emerging evidence suggests that adaptors can also act as essential cofactors for motor activation (Figure 1B). Recent work on a conserved family of motor adaptors, the Bicaudal D (BICD) proteins, demonstrated that they not only couple dynein motors to cargo but also stimulate processive dynein motility by promoting dynein interaction with its cofactor dynactin [19,20]. Here, we review the advances in the current understanding of how BICD adaptors control microtubule-based transport and discuss how this work has contributed to revealing general principles of motor regulation.

## Structure and Function of the Bicaudal D Family of Adaptor Proteins

Bicaudal D was initially identified in *Drosophila* through characterization of two dominant lethal maternal effect *BicD* mutations, which cause the transformation of the anterior segments of the embryo into posterior ones, which can result in a double abdomen, or bicaudal ('two-tailed') phenotype [21]. Subsequent intensive studies demonstrated that BicD regulates multiple steps in fly oogenesis and development by controlling mRNA transport and positioning of oocyte and photoreceptor nuclei ([22–27], reviewed in [28]). Analysis of the localization and function of *Drosophila* BicD has provided strong indications that the protein is involved in microtubule-dependent transport processes controlled by cytoplasmic dynein ([25,29], reviewed in [28,30]).

Biochemical support for the BICD–dynein interaction has initially come from work in mammalian cells [31]. Mammals possess two BicD orthologs, BICD1 and BICD2, as well as two more distantly related proteins named BICDR-1 and BICDR-2, which have a single as yet uncharacterized ortholog in flies (Figure 2A) [32]. All BICD family members contain several regions that can form coiled coil domains (Figure 2A), and structural work indicates that they adopt a rod-like shape [33,34]. The N-terminal two coiled coil domains of BICD2 as well as the N-terminal part of BICDR-1 bind to dynein and dynactin [31,32,35,36] (Figure 2A). Artificial tethering of the dynein–dynactin-binding N-terminal portion of BICD2 to different cargoes, such as mitochondria, peroxisomes, or different vesicles, induces their prompt dynein-dependent translocation towards microtubule minus ends [35–38].

The C-terminal parts of BICD molecules bind to different partners that determine their localization to cargoes (Figure 2A). The C termini BICD1, BICD2, BICDR-1, and *Drosophila* BicD bind to the small GTPase Rab6 [32,39–42]. Rab6 localizes to the Golgi apparatus and cytoplasmic vesicles, which were initially thought to mediate retrograde transport from the Golgi to endoplasmic reticulum (ER) [43], but were later shown to be exocytotic carriers [44]. Accordingly, BICD proteins can contribute to processes within the secretory pathway, such as Golgi organization and vesicle transport [32,40–42,45]. The functions of BICD1, BICD2, and BICDR-1 in the secretory pathway appear to be at least partially nonredundant. For example, BICD2 plays an essential role in the migration of cerebellar granule cells by affecting the extracellular matrix proteins produced by glia [46], while BICDR-1-dependent Rab6 vesicle transport is important in early neuronal development [32]. It should be noted that the redundancy between different mammalian BICD proteins has not been analyzed systematically, and the function of BICDR-2 is currently unknown. Interestingly, in the case of BICD2, the interaction with Rab6 is regulated during the cell cycle: in G2, BICD2 switches from Rab6 to the nucleoporin RanBP2, associates with the nuclear envelope, and recruits to it dynein and dynactin [47]. This BICD2 function, which seems not to be shared with BICD1, helps to separate and position the two centrosomes at the two sides of the nucleus to promote bipolar spindle formation around the chromosomes [47,48]. It also plays a role in pulling apart the acentriolar microtubule-organizing centers in mouse oocytes [49] and is important for regulating nuclear migration and mitotic entry in brain progenitor cells [50]. It is currently unclear to which other cargoes BICD adaptors can bind in mammalian cells; however, it was shown that BICD1 is involved in organizing microtubule arrays [51], contributes to the replication of cytomegalovirus by binding a virion tegument protein [52], and regulates the endosomal sorting of neurotrophin receptors in motor neurons [53].

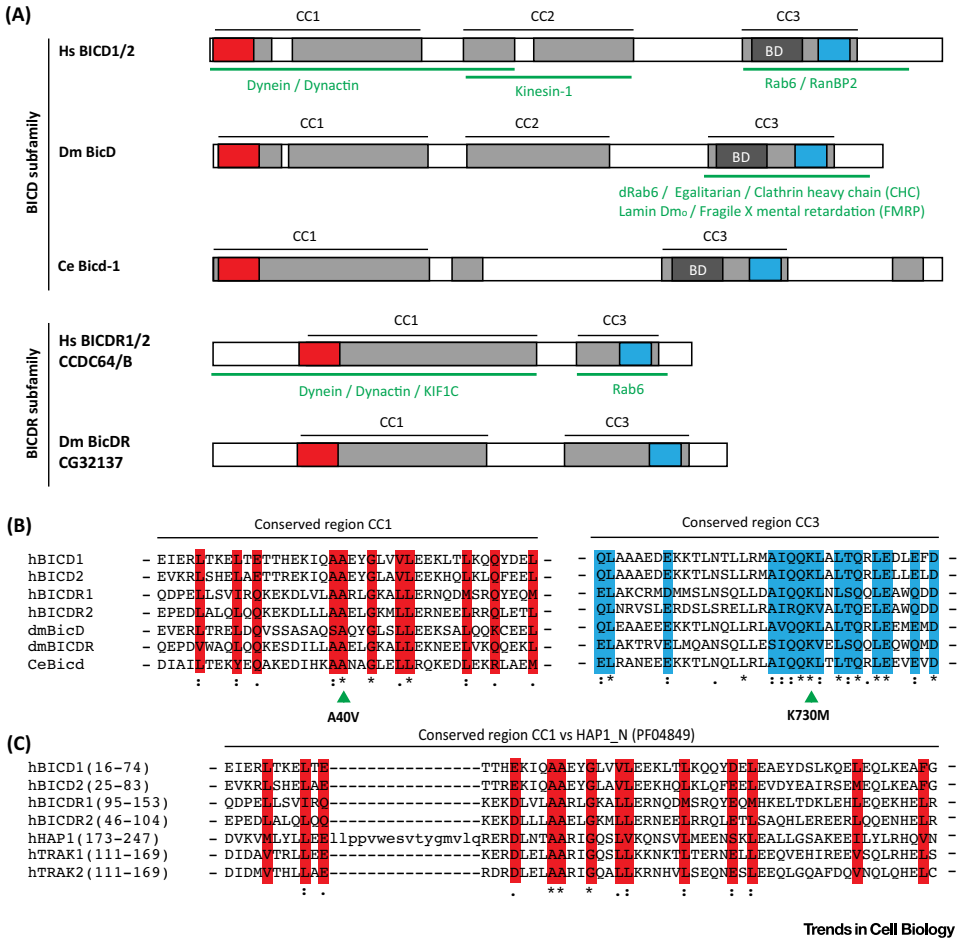


Figure 2. Schematic Structure of the Bicaudal D (BICD) Family Members. (A) Schemes of BICD family proteins from three different organisms are shown. The mammalian members include four homologs, BICD1, BICD2, BICDR1 (CCDC64), and BICDR2 (CCDC64B), while *Drosophila melanogaster* has two family members, BicD and BicDR (CG32137) and *Caenorhabditis elegans* has one family member, Bicd-1. As illustrated in the diagram, BICD family proteins are predicted to contain extensive α-helical coiled coil stretches (gray boxes). The family is characterized by the presence of an N-terminal coiled coil (CC1) domain with a conserved region (red box) and a C-terminal coiled coil (CC3) domain with a conserved region (blue box). The middle part of the proteins contains another coiled coil (CC2) region, which is poorly conserved in worm and in BICDR subfamily members. Moreover, the BICD subfamily contains a conserved region within the CC3 (named BICD domain; BD) that is not present in BICDR proteins. Note that despite the significant conservation, the structures of the C-terminal coiled coil domains of the mammalian and fly homologs might be different (see text for details). The green lines indicate the positions of the domains interacting with protein partners. Protein sequences with the following NCBI accession numbers were used for the drawings: BICD1 (NP_001705.2), BICD2 (NP_001003800.1), BICDR1 (NP_997194.2), BICDR2 (NP_001096645.1), BicD (NP_001260531.1), BicDR (NP_729935.1), and Bicd-1 (NP_501001). Abbreviations: Hs, *Homo sapiens*; Dm, *Drosophila melanogaster*; Ce, *Caenorhabditis elegans*. (B) Alignment of the amino acid sequences of BICD family members from different organisms. The BICD family contains an N-terminal coiled coil (CC1) domain with a highly conserved region (corresponding to the red box in A) around the sequence AAxxGxxVL. The *Drosophila* hypomorphic loss-of-function A40V mutation is part if this conserved region. Adjacent to the conserved alanine, mammalian BICD family members have an extra alanine residue in the conserved domain. The A→V mutation of the corresponding site in BICD2 and BICDR-1 reduces dynein and dynactin binding. In the C-terminal part of the protein, a third coiled coil (CC3) is present with a conserved region (corresponding to the blue box in A) around the sequence AIQQK(L/V)xL(T/S)QxLE. The *Drosophila* lethal K730M mutation in this region strongly interferes with the binding of several BICD-binding partners. (C) Alignment of the highly conserved region in the N terminus of human BICD family proteins and other human proteins with the HAP1_N conserved region (Pfam protein database: pf04849), found in several huntingtin-associated protein 1 (HAP1) homologs, such as TRAK1 and TRAK2. The following sequences were used for the alignment: UniProt accession numbers for human HAP-1 (P54257), TRAK1 (Q9UPV9), and TRAK2 (Q8IU62).

In flies, one of the most important partners of BicD is Egalitarian (Egl), which binds to mRNA localization elements of different developmentally important transcripts that are subject to dynein-mediated transport [27,29,54]. In addition, fly BicD interacts with the RNA-binding factor Fragile X mental retardation protein (FMRP) and clathrin heavy chain, and can thus participate in different pathways of mRNA and membrane transport, which are important for neuronal morphogenesis and synaptic function at the neuromuscular junction [55,56]. All existing data point to BICD proteins binding to one partner at a time. The binding sites for the partners can overlap extensively, like in the case of Rab6 and Egl [57], but can also show distinct features, like in the case of Rab6 and RanBP2 [58].

In addition to binding to partners, the C-terminal part of BICD has another function: by binding to the N-terminal portion of the molecule (Figure 3A), it attenuates the interaction of dynein and dynactin [31,35,36,58]. The simple model that emerges from all these studies suggests that BICD proteins act as motor tethers: when the C-terminal part of the protein binds to cargo, the N-terminal part becomes available to bind to dynein and induce minus end-directed transport [31]. This model is supported by the observation that mutating of the cargo-binding site in the BicD C terminus reduces dynein association [57].

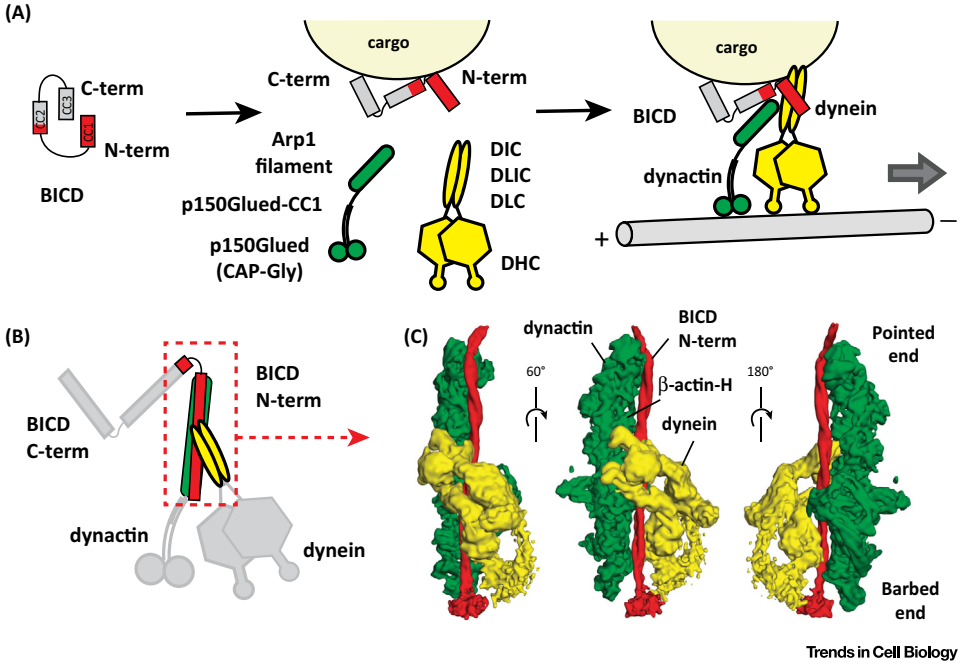


Figure 3. Recruitment of Dynactin by Cargo Adaptor Bicaudal D (BICD) Activates Dynein. (A) Schematic representation of the dynein–dynactin–BICD2 (DDB) complex on cargo. BICD2 is represented by three boxes, which correspond to three coiled coil regions (CC1, CC2, and CC3). The N-terminal region (CC1, red) contains an important part of the dynein/dynactin-binding domain. In this model, BICD2 may fold up, for example, as a result of interaction of CC1 and CC3, or CC2 and CC3. Only when BICD2 is bound to a cargo (such as Rab6 or Egalitarian) with its C-terminal domain, the N-terminal part becomes available for the interaction with dynein/dynactin. Dynein (yellow) and dynactin (green) are depicted as in Figure 1. The dynein motor is a complex of heavy (DHC), intermediate (DIC), intermediate light (DLIC), and light chains (DLC). Dynactin is composed of various subunits, including a short filament of actin-related protein 1 (ARP1) and the large dynactin subunit $p150^{Glued}$ with coiled coil region CC1 that can bind to DIC (not depicted), and a CAP–Gly domain, which associates with microtubules. The N-terminal portion of BICD2 (BICD2-N) promotes formation of a stable ternary complex with purified mammalian dynein and dynactin and shows highly processive minus end-directed motility. (B) Cartoon model of the dynein tail/dynactin/BICD2-N complex. (C) An 8.2 Å cryo-electron microscopy (cryo-EM) structure of the dynein tail/dynactin/BICD2N complex. The elongated BICD2-N molecule runs along the Arp1 filament and stabilizes its interaction with the dynein tail. Data are from [33].

Several lines of evidence indicate that this model is oversimplified. First, in addition to binding to dynein, BICD2 also interacts with kinesin-1 through its central coiled coil domain [44] (Figure 2A). Similarly, BICDR-1 binds to a kinesin-3 family member, KIF1C [32] (Figure 2A). So far, it has not been directly demonstrated that these interactions are sufficient to recruit kinesins to cargo, but it was shown that they could modulate the bidirectional cargo motility in a context-specific manner. For example, both kinesin-1 and dynein participate in controlling nuclear–centrosome interactions during mitotic entry, and removal of BICD2 blocks both motor activities at the nuclear envelope [47]. Along similar lines, studies of the transport of lipid droplets during *Drosophila* embryogenesis demonstrated that BicD plays a complex, temporary changing role in controlling the contribution of kinesin-1 and dynein to droplet transport [59].

Second, in many cases the interaction with BICD is not the only link between dynein and a particular cargo. For example, in addition to BICD, Rab6 can also directly interact with the dynactin large subunit p150$^{Glued}$ [39,60] and with dynein [61]. For both Rab6 and Egl, interactions with dynein light chains have been reported [62,63]. It should be noted, however, that structural work showed that dynein light chains cannot bind to dynein and other partners at the same time ([64], reviewed in [65]), making their role as potential cargo adaptors doubtful and the function of their interactions with Rab6 and Egl uncertain. In another example, the recruitment of dynein to the nuclear envelope before mitosis involves not only RanBP2 and BICD2 but also the nucleoporin Nup133, CENP-F, and another dynein partner, NUDE/EL (Box 1) [50,66]. A similar large protein complex including the homologs of dynein, BICD, NUDE, and kinesin-1 plays a role in nuclear migration in worms; however, in this system the complex is linked to a KASH domain protein UNC-83 and not to a nucleoporin [67]. These data show that dynein localization on a particular cargo requires formation of large protein complexes and does not necessarily depend on BICD proteins; for example, in the case of lipid droplets, there is no correlation between the amount of BicD and dynein on the droplets at different developmental stages [59]. Fly BicD is not strictly required for mRNAs to recruit motors and move in both directions on microtubules; however, together with Egl, BicD likely participates in recruiting dynein to RNA localization sequences and controlling the processivity of mRNP movement [68,69]. Taken together, these data suggest that the role of BICD adaptors in a particular transport process depends on the composition of the specific motor complex and likely involves not just motor tethering but also the regulation of its activity and processivity.

## The Mechanism of BICD as a Dynein Activator

How can adaptors such as BICD regulate motor activity and processivity? *In vitro* work with purified mammalian dynein has shown that individual motors do not move processively along microtubules [19,20,70], suggesting that additional factors regulate dynein motility. Dynactin was identified as a dynein cofactor required for almost every dynein function [71] (Box 1). Dynactin can increase dynein processivity when both complexes are attached to beads [72,73], but the interaction between the two complexes appears to be transient and regulated [74,75].

Interestingly, biochemical experiments showed that the N-terminal portion of BICD2 (BICD2-N) promotes formation of a stable ternary complex with purified mammalian dynein and dynactin [36]. Subsequent analysis of the behavior of these dynein–dynactin–BICD2-N (DDB) complexes by single molecule fluorescence microscopy *in vitro* revealed highly processive minus end-directed motility, with run lengths and speeds similar to those seen for dynein cargoes in cells [19,20]. Characterization of this complex by electron microscopy demonstrated that the elongated BICD2-N molecule runs along the Arp1 filament and stabilizes its interaction with the dynein tail [33,76] (Figure 3A,B). Stable binding of dynactin to dynein through BICD2-N can increase motor processivity by providing an additional microtubule-binding site through the N-terminal part of the dynactin large subunit p150$^{Glued}$ [19,20,73] (Box 1). However, this view is not without controversy, as there are cellular data pointing against the importance of the

### Box 1. Dynactin Is a Critical Dynein Cofactor

Microtubule minus end-directed cargo transport is driven primarily by cytoplasmic dynein acting in concert with its activator dynactin [4,71,119]. Dynein consists of a dimer of the motor domain containing heavy chain (DHC, encoded by the *DYNHC1H1* gene) and smaller subunits, including dynein intermediate chains (DIC), light intermediate chains (DLIC), and light chains (DLC). Dynactin is a 23 subunit complex with at least ten distinct polypeptides, such as the largest subunit p150$^{Glued}$ (DCTN1), dynamitin (DCTN2), and the actin-related protein 1 (Arp1), which forms a short filament (see Figure 3A in main text). The Arp1 filament is nine subunits long, of which one is β actin; it consists of two protofilaments and is capped by two different protein complexes [33]. At the barbed end, the CapZαβ heterodimer binds across both protofilaments and at the other end Arp11 binds to p62 (DCTN4), p25 (DCTN5), and p27 (DCTN6) to form the pointed end complex [33,76] (Figure I). The interaction between dynein and dynactin was reported to involve the first coiled coil region of p150$^{Glued}$ (CC1) and the N-terminal domain of DIC [120–122]. The binding between the two complexes is stabilized by the N-terminal region of BICD2 (BICD2-N) via interactions with the Arp1 filament and the dynein tail [20,35,36,80]. The DIC–p150$^{Glued}$–CC1 interaction was not directly visualized by cryo-electron microscopy (cryo-EM) [33,76], most likely due to the high flexibility in the two regions. Instead, the p150$^{Glued}$ region was found to interact to the Arp1 filament, similar to the BICD2-N association, suggesting that BICD2-N binding could free p150$^{Glued}$–CC1 to make contact with DIC2 and thus add an additional contact that stabilizes the dynein–dynactin complex [33,76]. The p150$^{Glued}$ subunit can bind microtubules via its N terminus that contains a conserved CAP–Gly domain (see Figure 3A in main text). This domain is dispensable for the activation of dynein motility [77,78], but is required for other functions such as recruitment of dynein to microtubule plus ends [123–125]. Besides dynactin and BICD2, several other regulatory proteins control dynein function and localization [4]. The best characterized dynein regulators are LIS1 (Lissencephaly 1), NDE1 (nuclear distribution protein E), and its paralog NDEL1 (NDE1-like). While LIS1 or NDE depletion often results in phenotypes similar to that of loss of dynein function, the precise mechanistic action of these regulators remains incompletely understood.

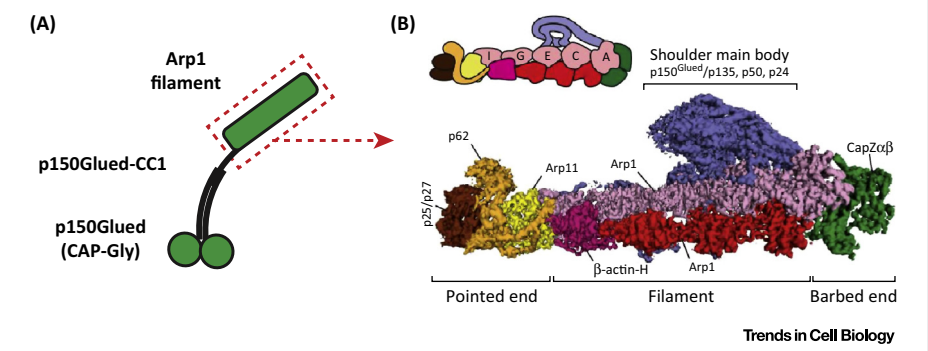


Figure I. Composition and Domain Structure of the Dynactin Complex. (A) Schematic representation of dynactin. The short filament of actin-related protein 1 (ARP1) forms the central core of the dynactin complex. The large dynactin subunit p150$^{Glued}$ (DCTN1) projects from the ARP1 filament, can interact with dynein via the coiled coil region CC1, and can bind to microtubules through a highly conserved cytoskeleton-associated protein glycine-rich (CAP–Gly) domain. (B) A 4.0 Å cryo-electron microscopy (cryo-EM) map of dynactin segmented and colored according to its components. Data are from [33]. The dynactin subcomplex at the pointed end of the ARP1 filament (purple/red) contains ARP11 (yellow), p62 (brown), p25 (brown), and p27 (DCTN6) (orange), while the barbed end subcomplex is composed of the CapZαβ heterodimer (green). The dynactin shoulder is composed of p150$^{Glued}$, a tetramer of p50/dynamitin and the p24 subunit (all blue).

microtubule-binding domain of p150$^{Glued}$ for dynein motility [77,78]. Another possibility is that the binding to dynactin and BICD2-N would alter the conformation of the dynein motor, for example, by promoting the separation of the two motor heads, which were reported to undergo an autoinhibitory interaction [79]. If the formation of a ternary complex with dynactin and an adaptor molecule can indeed have a direct impact on dynein activity, adaptors with distinct properties might have a different effect on the motor. This idea might help to explain how the BICD-related adaptor BICDR-1, which also stabilizes the dynein–dynactin association, can induce faster dynein-dependent vesicle movements than those dependent on BICD2 [80].

While the data described above are an important step forward in understanding dynein regulation, many questions remain. *In vitro* experiments predict that the DDB complexes should exhibit processive motility in cells. However, the opposite is true: overexpressed BICD2-N acts as a dynein inhibitor [31,81] by dissociating dynein and dynactin not only from cargoes but also

from microtubules [36]. By contrast, when the DDB is targeted to membrane organelles or protein complexes, it induces their dynein-based movement [35–38,54]. It is possible that dynein–dynactin complexes are present in multiple copies to confer motility in a cellular context, an idea supported by the estimation of the number of dynein motors on cargo [82]. The possibility that BICD proteins participate in macromolecular assemblies is also in line with work in flies: tethering of the N-terminal portion of BicD to mRNPs induces their minus end-directed translocation, but this process requires the endogenous full-length BicD [54].

It is also possible that additional dynein cofactors are involved. For example, Lissencephaly 1 (LIS1) is required for BICD-dependent transport processes both in flies and mammals [25,36,83]. The exact role of LIS1 in dynein motility is not yet fully understood; it binds to the dynein motor domain and increases its affinity for microtubules but does not prevent ATP hydrolysis (reviewed in [84]). *In vitro* work on budding yeast proteins showed that LIS1 acts as a clutch, which can uncouple the dynein's cycles of ATP hydrolysis and microtubule binding and unbinding [85,86]. Since yeast and mammalian dynein exhibit significant differences in their behavior [84], it would be interesting to know whether mammalian LIS1 has a similar effect. The affinity of LIS1 for dynein can be regulated by NUDE/EL proteins, which act in dynein recruitment pathways synergistically with the BICD adaptors [50,66,67]. How all these molecules work together is unclear, especially as NUDE and dynactin can undergo mutually exclusive interactions with the dynein complex [87]. Clearly, multicomponent *in vitro* reconstitution experiments will be needed to dissect these complexities.

## Insights into Structure–Function Relationships in BICD from Studies of Mutant Phenotypes

Genetic screens in *Drosophila* identified several dominant and recessive mutations in the *BicD* gene. The hypomorphic loss-of-function A40V mutation in the N-terminal part of the molecule maps to a conserved region (Figure 2B), which is likely to be involved in the interaction with dynein and dynactin, because mutation of the corresponding site in the BICD2 and BICDR-1 molecules leads to strongly reduced binding to these complexes [80] (Figures 2B,C and 4A). A lethal mutation in the conserved C-terminal region, K730M (Figures 2B and 4B), strongly interferes with the binding of several partners such as Rab6, Egl, and FMRP [55,57].

In contrast to these loss-of-function mutations, it is much more difficult to make sense of the gain-of-function mutations that cause the bicaudal phenotype. These mutations induce abnormal dynein-dependent accumulation of the mRNAs encoding the posterior determinant Oskar in the anterior part of the oocyte at the expense of the kinesin-mediated transport of these mRNAs to the posterior [21,27,63,88]. One of these BicD mutations, F684I, is located in the C-terminal coiled coil CC3 (Figure 4A); it does not affect cargo binding but rather increases the association of full-length BicD with dynein [57]. Structural analysis showed that the CC3 of fly BicD forms a parallel coiled coil, which contains a symmetric C-terminal part and a remarkably asymmetric N-terminal part that displays heterotypic coiled coil registry [57]. It is possible that this unusual structure is somehow involved in the conformational transition that leads to the loss of BicD autoinhibition upon cargo binding, for example, by switching from heterotypic to homotypic registry [57]. The F684I substitution might affect this transition, thus increasing the binding of BicD and the associated mRNAs to dynein [57]. Surprisingly, the recently determined crystal structure of BICD1 CC3 revealed a parallel coiled coil with a completely homotypic registry [58]. Additional work is thus needed to understand the conformational transitions in full-length BICD proteins that are associated with cargo and motor binding and relief of autoinhibition. Such work will likely help to understand the second dominant *BicD* mutation, which is located in the middle of the molecule (Figure 4A).

Recently, multiple mutations have also been identified in one of the two human BICD-encoding genes, *BICD2*, in patients suffering from an autosomal dominant form of spinal muscular atrophy

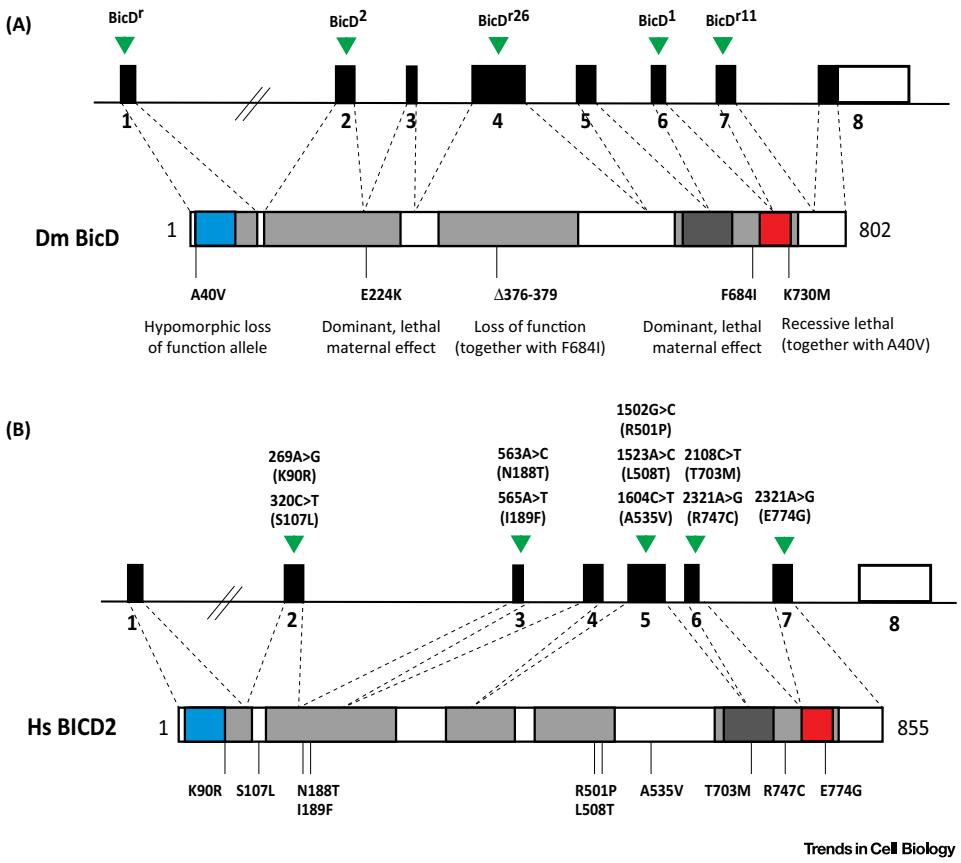


Figure 4. Schematic Representation of *Drosophila BicD* and human BICD2 Mutations. (A) Schematic representation of the *Drosophila BicD* gene and protein showing the location of the coiled coil domains, the position of *BicD* mutations, the corresponding amino acid substitutions, and their phenotypes. (B) Schematic representation of the human *BICD2* gene and protein (the 855 amino acid isoform is depicted). The position of the mutations found in patients with SMALED2 is shown above the BICD2 locus. All SMALED2 mutations are heterozygous and lead to single amino acid substitutions. The most common variant reported is the S107L mutation. Abbreviation: BicD and BICD, Bicaudal D; SMALED2, spinal muscular atrophy (SMA) with lower extremity predominance (LED) 2.

(SMA) with lower extremity predominance (LED) (SMALED2; OMIM 615290), also known as dominant congenital spinal muscular atrophy (DCSMA) [89–94] (Box 2). All these mutations are single amino acid substitutions that are scattered throughout the BICD2 protein (Figure 4B). The pathology in these patients is probably caused by the impairment of transport processes in motor neurons. The essential role of BICD adaptors for different neuronal functions, such as transport, is strongly supported by studies in both vertebrates and invertebrates [46,50,53,55,56,95]. Biochemical evidence suggests that some SMALED-associated mutations can affect the binding of BICD2 to one of the partners: the S107L mutant might increase dynein–dynactin binding, and the Q774G substitution may decrease the interaction with Rab6 [89,94]. Moreover, overexpression of various BICD2 mutants disrupts Golgi morphology [89,91], one of the hallmarks of altered dynein activity [96]. Importantly, heterozygous mutations in the cytoplasmic dynein heavy chain, *DYNC1H1* (SMALED1; OMIM: 158600), cause the same patient phenotype as *BICD2* mutations [97–103] (Box 2). The *BICD2* mutations thus likely perturb dynein-dependent transport in neurons. The connection to kinesin-1 could also play a role: some mutations, such as R501P and L508T, are located in the central, kinesin-1 binding part of the molecule [44]. Compatible with this notion, mutations in the kinesin-1 family member KIF5A are associated with familial hereditary spastic paraplegia [104], and KIF5C knockout mice show

### Box 2. Dominant Congenital Spinal Muscular Atrophy Is Caused by Heterozygous Mutations in the *BICD2* Gene

Spinal muscular atrophy (SMA) is a hereditary neuromuscular disorder characterized by degeneration of spinal cord motor neurons resulting in muscle weakness [126]. The most common form of SMA (SMA1; OMIM: 253300) shows autosomal recessive inheritance and is due to mutations in the survival motor neuron 1 (*SMN1*) gene on chromosome 5q [127]. Absence of SMN1 is partially compensated for by SMN2, which may produce sufficient SMN protein to allow for relatively normal development in cell types other than motor neurons [128]. There are numerous other forms of SMA that share certain characteristics SMA1 (referred to as non-5q SMA); however, they are genetically distinct and often affect different subsets of neurons and muscles, including autosomal dominant forms of the disease [126]. Spinal muscular atrophy, lower extremity predominant (SMALED), also known as dominant congenital spinal muscular atrophy (DCSMA), collectively describes a group of patients with non-5q SMA who are characterized by early childhood degeneration of lower spinal cord motor neurons resulting in muscle weakness, which often leads to significant mobility impairment. Mutations in the heavy chain of cytoplasmic dynein (*DYNC1H1*) were the first to be identified as a cause of SMALED type 1 (SMALED1; OMIM:158600) [97–103]. More recently, mutations in BICD2 have been identified in SMALED type 2 (SMALED2; OMIM 615290) ([89–94]; for a recent review, see [129]). Mutations in DYNC1H1 and BICD2 typically cause early-onset lower limb predominant weakness, with the same degree of disability in later life suggesting a nonprogressive disorder of motor neuron development. These features are consistent with SMALED1/2 being a 'developmental' form of SMA. However, it remains unknown whether mutations in *DYNC1H1* and *BICD2* affect the primary development and/or early survival of the subpopulation of lower motor neurons. Moreover, the precise molecular mechanism underlying SMALED is yet unknown, but the clinical similarity between the *BICD2* and *DYNC1H1* patients strongly suggests that dynein function is critical for motor neuron development and survival. Consistently, genetic defects associated with motor neuron diseases and other neurodevelopmental disorders have been found in other adaptors that regulate dynein function, including the dynactin subunit p150$^{Glued}$, LIS1, and NDE1 [130]. For instance, p150$^{Glued}$ mutations (DCNT1; OMIM: 601143) are associated with neurodegenerative disorders such as slowly progressing lower motor neuron disease [hereditary motor neuronopathy type VIIB (HMN7B); OMIM: 607641] and parkinsonian disorder (Perry syndrome; OMIM: 168605) [131,132]. In the future, it will be important to find out the various mechanisms by which disease-related mutations in adaptor proteins disrupt dynein function in the developing nervous system.

### Outstanding Questions

What is the precise architecture of the cargo–adaptor–motor complexes?

What is the exact molecular mechanism underlying dynein activation by dynactin?

What are the regulatory mechanisms controlling the interactions between BICD and related adaptors with motors and cargo?

What is the structural basis of conformational transitions in BICD and related adaptors that control motor–cargo attachment?

Why do missense mutations in the ubiquitously expressed BICD2 and dynein lead to motor neuron disease in humans?

Why does BICD2 loss of function lead neuronal migration defects in mice?

loss of motor neurons [105]. BICD2 mutations might also cause changes in the conformational transitions associated with autoinhibition and its relief, as proposed earlier for the fly BicD.

The exact molecular mechanisms underlying SMALED are not yet clear. BICD2 mutations are linked to SMA with dominant inheritance, but it is currently not known whether the resulting alterations in BICD2 protein lead to gain-of-function or dominant-negative loss-of-function effects, or a loss of function combined with haploinsufficiency. Both short hairpin RNA (shRNA)-mediated depletion of BICD2 in the developing neocortex and BICD2 knockout mice show severe neuronal migration defects [46,50]. By contrast, malformations of cortical development have so far not been described for patients with *BICD2* mutations, although such developmental defects were linked to *DYNC1H1* mutations [106]. These data might suggest that the BICD2-associated phenotypes linked to SMALED might represent specific gain-of-function alterations in BICD2, to which motor neurons are particularly sensitive.

## Concluding Remarks

BICD adaptors are not unique in their mode of action. It was shown that in addition to BICD2, other dynein adaptors, such as Rab11–FIP3, hSpindly, and Hook3, also form tripartite complexes with dynein and dynactin and thus promote processive minus end-directed motility of dynein motors [20]. These results suggest a universal model of activation dynein motility that requires the simultaneous binding of the general activator dynactin and selective motor–adaptor proteins present on particular cargo. Other coiled coil motor adaptors, such as HAP1 and TRAK1/2, also bind to dynein and dynactin [15,107,108], share some similarity with the N-terminal dynein–dynactin-binding region of BICD (Figure 2C) and might act in a similar manner. Interestingly, HAP1 and TRAKs, as well as their fly ortholog Milton also share the ability of BICD2 to interact not only with dynein but also with kinesin-1 [15,109–111]. However, in contrast to BICD proteins, which predominantly control the dynein-dependent minus end-directed transport, HAP1, TRAK1, and Milton are highly important for the plus end-directed motion [15,109,112,113]. For the TRAK proteins, it was shown that the ability to interact with dynein

and kinesin-1 is regulated by their self-folding, emphasizing the importance of conformational transitions in the adaptors for controlling bidirectional motility [15].

In spite of significant progress, many important questions remain (see Outstanding Questions). First, to gain insight into how motor–adaptor assemblies control cargo motility, we need to understand much better the architecture of these macromolecular complexes. Second, we need to find out how these complexes are regulated. Several kinases, including Polo and GSK-3β, interact with BICD adaptors both physically and functionally [51,114–116]; however, it is not yet known how phosphorylation regulates BICD conformation or its association with partners. Interestingly, the mutation of at least one phosphorylation site, S103, was shown to modulate BicD activity in *Drosophila* [117]. A combination of detailed mutational studies with multicomponent *in vitro* reconstitutions and structural work will be required to achieve these goals.

Another important challenge is to understand the cell type-specific functions of these molecules. Why do missense mutations in the ubiquitously expressed *BICD2* gene lead to pathology that manifests itself predominantly in motor neurons? Interestingly, a recent study showed that low chaperone activity in motor neurons is as an important factor associated with their vulnerability to misfolded proteins [118]. It is thus possible that protein folding-related mechanisms play a role in motor neuron pathology induced by mutant BICD2. Regardless of the underlying mechanism, it is important to note that the similarities between the phenotypes induced by *BICD2* and *DYNC1H1* mutations in SMA have highlighted dynein-mediated cargo trafficking as a key pathway in motor neuron development, function, and survival. The studies of this evolutionary conserved pathway thus provide a promising avenue for discovering therapeutic targets for motor neuron disorders and other neurodegenerative diseases.

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