Bicaudal-D and its role in cargo sorting by microtubule-based motors

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Abstract

Many cytoplasmic cargoes are transported along microtubules using dynein or kinesin molecular motors. As the sorting machinery of the cell needs to be tightly controlled, associated factors are employed to either recruit cargoes to motors or to regulate their activities. In the present review, we concentrate on the BicD (Bicaudal-D) protein, which has recently emerged as an essential element for transport of several important cargoes by the minus-end-directed motor cytoplasmic dynein. BicD was proposed to be a linker bridging cargo and dynein, although recent studies suggest that it may also have roles in the regulation of cargo motility. Here we summarize the current knowledge of the role that BicD plays in the transport of diverse cellular constituents. We catalogue the molecular interactions that underpin these functions and also highlight important questions to be addressed in the future.

Introduction

Sorting of many cargoes within cells is mediated by microtubule-based transport using dynein or kinesin molecular motors. Therefore the recruitment of cargoes and activities of motor complexes have to be tightly regulated to ensure proper spatial–temporal organization of the cell. Recruitment of motors to cargoes is often indirect, involving associated adaptor proteins and cofactors [1]. In the present review, we concentrate on the BicD (Bicaudal-D) protein that has been reported to play a vital role in the sorting of a subset of cellular constituents as part of dynein transport complexes.

The BicD protein

BicD was originally identified in Drosophila by the characterization of two dominant maternal-effect mutations [2]. Its name derives from the large proportion of mutant embryos with an anterior to posterior transformation (the word ‘bicaudal’ means ‘two tails’), which is likely to be related to the function of the protein in mRNA localization (see below). BicD is evolutionarily highly conserved in metazoan animals (Figure 1). While there is only one isoform of BicD in invertebrates, two isoforms (BicD1 and BicD2) exist in mammals, which are able to partially compensate for each other’s function [3].

BicD contains extensive heptad repeats forming several predicted coiled-coil domains (Figure 1). While no high-resolution structure of BicD has been published to date, electron microscopy of recombinant BicD revealed a comma-shaped structure with thin- and thick-rod-shaped regions connected by a flexible linker. This was interpreted to be a parallel dimer in which the N-terminal half of each molecule forms the thin rod, whereas the two C-terminal coiled-coil domains fold back on each other to form the thick rod [4].

BicD has a widespread cytoplasmic distribution [5,6] and is also enriched towards the minus-ends of microtubules [3,5,7]. Similarities between the phenotypes caused by BicD loss-of-function mutations and by microtubule disruption, together with the genetic requirement for BicD in the localization of several cargoes during Drosophila development, led to the speculation that BicD participates in dynein motor transport [8–11].

However, the first concrete link between BicD and dynein transport was established in mammalian cells, where BicD was found to be required for dynein-mediated Golgi–ER (endoplasmic reticulum) trafficking of Rab6-positive vesicles and to physically interact with components of the dynein–dynactin motor complex [5,12,13]. The N-terminal two thirds of BicD were found to associate in extracts with dynein intermediate chain [3,5] and the C-terminal part was shown to interact directly with p50 dynamin [5] (Table 1).

A molecular model of BicD acting as a linker between cargoes and the dynein complex was also established in mammalian cells. The C-terminal region, which binds Rab6, was proposed to be responsible for recruitment of cargoes, whereas the N-terminal part was thought to mediate functional association with the dynein motor. In a very informative study, the N-terminal two-thirds of mammalian BicD was found to be sufficient to stimulate dynein-mediated transport of artificially tethered cytoplasmic organelles [6]. In contrast, the full-length BicD was unable to support efficient transport of tethered cargoes [6]. It was proposed that the intramolecular interaction observed in BicD by electron microscopy and biochemical studies [4,5] has an autoinhibitory effect, which can be released after cargo binding to the C-terminal region.

Key words: Bicaudal-D (BicD), cargo transport, dynein, microtubule-dependent transport, mRNA localization, Rab6 vesicle.

Abbreviations used: BicD, Bicaudal-D; Dlc, dynein light chain; Egl, Egalitarian; ER, endoplasmic reticulum; GSK3β, glycogen synthase kinase 3β; TGN, trans-Golgi network.

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Figure 1 | BicD is an evolutionarily conserved coiled-coil protein

Drosophila BicD (dBicD, P16568) coiled-coil domains (black bars) were predicted by MARCOIL1.0 (probability >90%) [49] (top). The Drosophila BicD (dBicD) sequence was aligned with human BicD1 (Q96G01) and BicD2 (Q8TD16) using ClustalW (version 1.4). The percentage of identity in different segments of corresponding dBicD sequence is graphically displayed in shades of grey (middle). Drosophila mutant alleles described in the text causing dominant (closed arrows) or recessive loss-of-function (open arrowhead) phenotypes are also indicated (bottom).

Table 1 | A summary of known molecular interactions of BicD

<table>
<thead>
<tr>
<th>Interacting partner</th>
<th>BicD protein</th>
<th>BicD fragment</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polo kinase</td>
<td>dBicD</td>
<td>120–350</td>
<td>[18]</td>
</tr>
<tr>
<td>Kinesin 1 heavy chain</td>
<td>BicD2</td>
<td>336–595</td>
<td>[35]</td>
</tr>
<tr>
<td>GSK3β</td>
<td>BicD1</td>
<td>437–617</td>
<td>[3]</td>
</tr>
<tr>
<td>Lamin Dm0</td>
<td>dBicD</td>
<td>668–738</td>
<td>[4]</td>
</tr>
<tr>
<td>Rab6a, Rab6b</td>
<td>BicD1</td>
<td>673–803</td>
<td>[12,13,50]</td>
</tr>
<tr>
<td>Drab6</td>
<td>dBicD</td>
<td>699–772</td>
<td>[37]</td>
</tr>
<tr>
<td>p50 dynamin</td>
<td>BicD2</td>
<td>706–810</td>
<td>[5]</td>
</tr>
<tr>
<td>Dynein intermediate chain</td>
<td>BicD2</td>
<td>1–271</td>
<td>[3,5]</td>
</tr>
<tr>
<td>Dynein-dynactin complex</td>
<td>BicD2</td>
<td>1–575</td>
<td>[35]</td>
</tr>
<tr>
<td>Egalitarian</td>
<td>dBicD</td>
<td>Full-length</td>
<td>[7]</td>
</tr>
<tr>
<td>Ninein</td>
<td>BicD1</td>
<td>Full-length</td>
<td>[3]</td>
</tr>
<tr>
<td>Nek8</td>
<td>BicD2</td>
<td>Full-length</td>
<td>[46]</td>
</tr>
<tr>
<td>Lipid droplets</td>
<td>dBicD</td>
<td>Full-length</td>
<td>[41]</td>
</tr>
</tbody>
</table>

The data strongly support such a model, although confirmation of this scenario in other organisms is still pending.

Cargoes transported by the BicD-dependent machinery

In the following sections, we discuss the characterized roles of BicD in dynein-mediated transport of cellular cargoes.

Localization of mRNAs

BicD is essential for correct specification of the Drosophila oocyte. This is likely to be linked, at least partially, to a requirement for the protein in microtubule-dependent accumulation of oocyte-specific mRNAs [8]. Although BicD-null mutants fail to differentiate an oocyte, experiments in which BicD function is perturbed only partially have shown that the protein also plays a role in mRNA localization.
during later stages of oogenesis [10]. BicD is required for the oocyte growth and is involved in the transport of transcripts from nurse cells to the interconnected transcriptionally silent oocyte, where minus-ends of microtubules are nucleated, consistent with a dynein-related function [10,14]. In mid-oogenesis, the correct subcellular localization of polarity determinants within the oocyte is also dependent on BicD activity [10]. This includes anteriorly localized bicoid and K10 mRNAs, dorso-anterior gurken mRNA and posterior oskar mRNA. The bicaudal phenotype observed in two dominant BicD alleles (BicD¹ and BicD²) is likely to be due to ectopic anterior accumulation of oskar mRNA in these mutants [15]. Interestingly both dominant mutations, which map to single amino acid substitutions [16], reside in different parts of the protein (Figure 1) and the molecular basis of the dominant effect is still unclear.

The role of BicD in mRNA transport in the oocyte appears to be intimately linked with another protein, Egl (Egalitarian). Egl co-immunoprecipitates with BicD [7], egl- and BicD-null mutants exhibit very similar defects [8,17,18], and both proteins interdependently co-localize throughout the whole oogenesis [7,19].

The BicD- and Egl-dependent RNA transport machinery is also used in embryogenesis. These two proteins play an essential role in the dynein-mediated apical localization of pair-rule transcripts in the syncytial blastoderm [20,21], which facilitates the nuclear uptake of the transcription factors they code for [22]. This machinery is also used for the apical localization of inscutable mRNA in neuroblasts [23] and wingless mRNA in epithelial cells [22,24]. The minus-end-directed localization of mRNA along microtubules depends on cis-acting RNA localization elements [25–29]. Where tested, the same elements are able to mediate minus-end-directed transport both in the oocyte and in the embryo [20,29], further supporting analogous transport mechanisms in both systems.

The mechanism of Egl- and BicD-dependent apical RNA localization in the embryo has been further scrutinized by the injection of in vitro synthesized fluorescent mRNAs [20,21]. Egl and BicD are recruited to injected transcripts containing localization elements, but not to control RNAs. They are then co-transported with RNA particles in a dynein-dependent manner towards the minus-ends of microtubules nucleated in the apical cytoplasm [21,30]. Analogously in the oocyte, in vitro synthesized bicoid and gurken mRNAs injected into nurse cells have been shown to recruit Egl and BicD, move along a directed path towards the ring canals and finally localize into the oocyte similarly to the endogenous transcripts [14].

The apical transport efficiency of mRNAs correlates with cytoplasmic levels of Egl, BicD and Dlc (dynein light chain), and these proteins are recruited differently to different localization signals [30]. Interestingly all mRNAs undergo short bidirectional movements in the cytoplasm, which are strongly reduced in both directions by interfering with dynein activity. However, removal of Egl or BicD function, or RNA localization elements, only abolishes the relatively long minus-end-directed runs. This implies that molecular motors may be recruited to all mRNAs independently of Egl, BicD and localization signals and that these factors may be needed to augment minus-end movements of localizing transcripts [30]. It will be interesting to determine if this could be achieved by regulating copy number of the dynein motor per RNA particle or through higher-order changes in dynein/dynactin activity.

Egl has been shown to directly bind Dlc [31], illuminating a further potential link of the complex to dynein in addition to that mediated by BicD. However, neither Egl nor BicD contains a characterized RNA-binding motif and therefore additional factors may be needed to link the complex to mRNA localization elements. The localization elements characterized so far [25–29] do not share any obvious consensus signals, and so far no protein has been identified specifically binding localization elements from different transcripts. It is therefore not clear whether a shared recognition machinery exists or whether different transcripts are linked to BicD-containing transport complexes using dedicated sets of recognition factors [32].

**Vesicle transport from the Golgi to the ER**

In COS-1 cells, BicD2 distribution largely overlaps with γ-adaptin, a marker for clathrin-coated vesicles at the TGN (trans-Golgi network) [5]. Further evidence demonstrated that BicD is involved in the COPI (coatamer protein I)-independent retrograde transport of vesicles from the Golgi to the ER [12].

The small GTPase Rab6, which plays an essential role in this transport step, binds mammalian BicD proteins [12,13,33]. BicD and Rab6 largely overlap at the Golgi apparatus and on vesicle-like structures in the cytoplasm, and co-localize on a well-characterized Rab6-dependent retrograde cargo, Shiga toxin subunit B [12]. Rab6 binds BicD in a GTPase-dependent manner through the BicD C-terminus [12,33] to link Golgi vesicles to dynein and dynactin. Tethering BicD on to vesicles requires Rab6, whereas recruitment of the dynein–dynactin complex on to Rab6-positive vesicles requires BicD [5,12,13], indicating that BicD facilitates the recruitment of Rab6-positive vesicle cargoes on to the motor complex. Additionally, a direct association between Rab6 and the dynactin subunit p150Glucl was also observed [13], so this interaction could promote the recruitment of the motor complex to the TGN. Collectively, these results suggest that trans-Golgi vesicles are BicD/Rab6-dependent cargoes. Consistent with this notion, Rab6-positive vesicles undergo microtubule-dependent movements [12,34]. This motility was substantially altered in cells overexpressing the C-terminus of BicD [12], presumably because this domain competes with the endogenous protein for Rab6 binding.

Although the current available evidence mostly links BicD’s role in dynein, the plus-end-directed motor kinesin-1 was also shown to interact directly with BicD2 [35]. This interaction may stimulate microtubule-based transport of Rab6-positive exocytic vesicles to the cell periphery where plus-ends of microtubules are abundant [35]. Thus BicD’s role may not be restricted to the minus-end-directed...
motor dynein. Below we discuss more evidence for BicD functioning with kinesin-1 during bidirectional lipid droplet transport in Drosophila.

The interaction between BicD and Rab6 is conserved from Drosophila to mammals, and binding between Drosophila Rab6 and BicD is also executed through the C-terminal end of BicD [36,37]. These studies also revealed a role of Rab6 in patterning the Drosophila oocyte. This may reflect an indirect requirement of the membrane trafficking pathway in polarizing the cytoskeleton or a direct role of Rab6 in mRNA transport complexes. At least in the case of Egl-dependent mRNA transport, Rab6 does not appear to play a direct role as strong hypomorphic Rab6 mutant embryos do not alter the efficiency of apical transport of injected mRNAs in blastoderm embryos [37a]. This suggests that the complexes that BicD forms with Rab6 are functionally independent of those it forms with Egl.

**Migration of nuclei**

The dynein-driven translocation of nuclei along microtubules is essential for cell polarization during many processes [38]. Lis1 (Lissencephaly 1) functions with BicD in nuclear migration in the ovary and the developing eye imaginal disc in Drosophila [9]. The phenotype of mislocalized nuclei in eyes is similar to that observed in flies with a mutant dynactin subunit [39], suggesting that it is due to defects in dynein-dependent transport of nuclei. The Ste20-like kinase Misshapen, a fly homologue of the vertebrate Nck-interacting kinases, functions together with BicD and dynein in driving nuclear migration to the apical side of photoreceptor in the eye disc, and an increased level of BicD phosphorylation was induced by overexpression of Misshapen in cell culture [40]. This suggests that the nuclear transport process may be modulated by BicD phosphorylation, although the potential molecular consequences of this modification are currently unknown.

**Lipid droplet transport**

Interesting insights into BicD’s role in microtubule transport have come from recent studies of bidirectional movements of lipid droplets in the Drosophila blastoderm embryo [41]. BicD was found on lipid droplets, which display movements in both the minus- and plus-end directions. These movements are driven by dynein and kinesin-1 respectively, which are simultaneously bound to the cargo [42]. Movements in both directions are sensitive to lowered BicD levels, suggesting that BicD may also affect kinesin-dependent transport, reminiscent of the situation described above during movement of Rab6-positive exocytic vesicles. The role of BicD goes beyond simply tethering motors to droplets, given that the amount of dynein and kinesin on droplets remains constant, whereas BicD gradually dissociates from droplets during development. In this situation, as in the case of embryonic mRNA transport, BicD may not be an obligatory linker but may affect modulation of motile properties in response to a specific cargo.

**Other potential cargoes for BicD**

The protein ninein is known to play an important role in anchoring of microtubule minus-ends at the centrosome [43]. BicD1 associates with ninein in mammalian cells and is required for its dynein/dynactin-mediated localization to the centrosome [3].

The proper localization of the cell-cycle-regulatory kinase Polo in the Drosophila oocyte requires BicD, Egl and the dynactin component dynamitin. Conversely, Polo is required for the activation of BicD-dependent transport from the nurse cells to the oocyte. BicD interacts with Polo in the yeast two-hybrid system and the two proteins co-immunoprecipitate from ovary extracts [18].

Lamins are nuclear-specific intermediate filament proteins. They play significant roles in the spatial organization of the cell nucleus [44]. The Drosophila BicD C-terminal coiled-coil domain interacts with the B-type lamin Dm0 in the yeast two-hybrid assay [4]. This interaction is specifically disrupted by a point mutation in the BicD dominant allele, although its physiological relevance is still unknown.

BicD1 localizes to inclusions of Chlamydia bacteria in infected mammalian cells [45]. This recruitment is mediated by the C-terminus of BicD and is independent of microtubules and Rab6. As the association of BicD depends on Chlamydia gene expression, it will be interesting to see if a bacterial protein can directly contact BicD in order to exploit the cellular motor machinery.

**Future prospects**

We have briefly reviewed here the current understanding of BicD’s roles in the transport of different cargoes and indicated a number of molecular interactions that appear to contribute to these processes (summarized in Table 1). Obviously there are still many open questions about this interesting molecule to be addressed, some of which have already been highlighted above.

As BicD is a highly conserved protein, it is likely that at least some of its interactions are maintained across species, although this needs to be confirmed. The direct link of BicD to many cargoes such as mRNA, nuclei and lipid droplets still needs to be identified and new potential cargoes for BicD-mediated transport should be investigated. It will be interesting to find whether other BicD cargoes associate, either directly or via adaptors, with the BicD C-terminal domain (as is the case for Rab6) and whether co-operative or competitive binding regulates the allocation of the BicD machinery between different cargoes. Further analysis of the molecular link between BicD and dynein/dynactin components is equally important. To date, the only direct contact that has been physically mapped is between the BicD C-terminal domain and dynamitin [5], whereas the N-terminal domain was suggested to be sufficient for dynein-mediated transport of tethered cargoes in vivo [6].

The precise function BicD plays within the transport complex should also be further scrutinized. The model of BicD as a passive linker between cargo and the dynein motor

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complex has been challenged by recent evidence from studies of mRNA and lipid droplet motion [30,41], which indicates that BicD is not obligatory for recruitment of dynein to these cargoes. Therefore a more complex role of BicD in regulating motion by facilitating the interaction of dynein, dynactin and kinesin motor complexes may emerge in the future.

The regulation of BicD activity at the post-translational level should be further investigated. GSK3β (glycogen synthase kinase 3β) [3] and Nek8 [46] can phosphorylate human BicD1 and BicD2 respectively in vitro, and Polo [18] and Misshapen [40] kinases may phosphorylate the Drosophila orthologue. The BicD female sterile allele BicD<sup>stam</sup>, with a single amino acid substitution A40V, is associated with hypophosphorylation of BicD [8]. Recently, serine phosphorylation of BicD was systematically examined and the findings were consistent with multiple, functionally redundant phosphorylation events [47]. As the kinase activity of GSK3β is essential for complex formation between BicD1 and dynein intermediate chain in vitro [3], it seems likely that the phosphorylation state of BicD regulates the assembly of the transport complex. It will be interesting to determine whether there is a link between BicD phosphorylation and overcoming the autoinhibitory interaction of the protein.

Many BicD phenotypes in the early Drosophila oocyte can be linked directly to dynein-based transport of mRNA. However, both BicD and Egl are also required for the initial restriction of meiosis into a single cell in early oogenesis and this process is not disrupted by microtubule-depolymerizing drugs or dynamin mutants [48]. This implies that Egl and BicD may have roles outside of microtubule motor-dependent transport. Finding what these roles are is likely to depend on identifying new physical or genetic interactors of these proteins.

Finally, there is evidence of a role for BicD and Egl in organizing the microtubule network in the germanium of the egg chamber [19]. A proposed role of BicD in nucleation of microtubules in the early oocyte [17] may now be reinvestigated in the light of the more recent molecular insights from mammalian cells [3].

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References


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