Evidence for cooperative interactions between the two motor domains of cytoplasmic dynein

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Cytoplasmic dynein is a force-transducing ATPase that powers the movement of cellular cargoes along microtubules. Two identical heavy chain polypeptides (> 500 kDa) of the cytoplasmic dynein complex contain motor domains that possess the ATPase and microtubule-binding activities required for force production [1]. It is of great interest to determine whether both heavy chains (DHCs) in the dynein complex are required for progression of the mechanochemical cycle and motility, as observed for other dimeric motors. We have used transgenic constructs to investigate cooperative interactions between the two motor domains of the *Drosophila* cytoplasmic dynein complex. We show that 138 kDa and 180 kDa amino-terminal fragments of DHC can assemble with full-length DHC to form heterodimeric complexes containing only a single motor domain. The single-headed dynein complexes can bind and hydrolyze ATP, yet do not show the ATP-induced detachment from microtubules that is characteristic of wild-type homodimeric dynein. These results suggest that cooperative interactions between the monomeric units of the dimer are required for efficient ATP-induced detachment of dynein and unidirectional movement along the microtubule.

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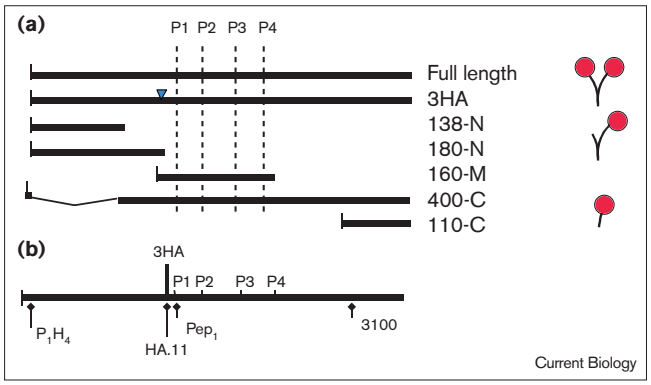
Results and discussion

We generated transgenic lines of *Drosophila melanogaster* that coexpress discrete domains of the previously characterized DHC [2] together with endogenous full-length DHC (Figure 1a). The carboxy-terminal ~400 kDa of DHC constitutes the globular motor domain and the amino-terminal region is present in the tail or stalk domain of the motor complex [3]. The amino-terminal fragments are expected to form a single-headed heterodimer by associating with the native DHC [4]. We first showed that certain truncated DHC polypeptides were able to assemble into a dynein motor complex. Dynein complexes were immunoprecipitated using antibodies that recognize epitopes present in the full-length DHC but that are missing from the truncated DHC fragments (Figure 1b). The immunoprecipitates were subsequently analyzed on immunoblots using an antibody that recognizes an epitope common to both the intact and truncated DHC polypeptides. The results show that an amino-terminal 180 kDa fragment, 180-N, was co-immunoprecipitated in a complex together with the intact endogenous DHC (Figure 2a). As a control, we showed that an antibody against the influenza hemagglutinin (HA) epitope will precipitate 180-N in a complex with an HA-tagged, full-length DHC (Figure 2b). In addition, a smaller 138 kDa amino-terminal fragment, 138-N, was also immunoprecipitated in a complex with the HA-tagged DHC (Figure 2c), whereas a carboxy-terminal 400 kDa DHC fragment (400-C) that comprises the globular motor domain did not (Figure 2d).

The assembly of the amino-terminal DHC fragments into a dynein complex was further demonstrated by cosedimentation experiments. The full-length DHC migrated in a 19–20S complex on sucrose density gradients (Figure 2e). The 400-C fragment lacked the amino-terminal 138 kDa of the tail domain, failed to co-immunoprecipitate with the full-length DHC (Figure 2d), and did not cosediment with the native dynein complex (Figure 2e). In the presence of 138-N, however, the sedimentation of intact DHC was altered. The 138-N fragment migrated on the gradient as a broad peak between 11S and 19S, consistent with the formation of heteromeric complexes with the intact endogenous DHC. The broadening of the peak is also consistent with the formation of heterodimeric dynein complexes of intermediate density (Figure 2f). Similar results were obtained with 180-N (data not shown). Furthermore, the mobility of 138-N compared with 400-C (11–19S versus ~13S) is not consistent with migration of 138-N as a monomer. These two lines of evidence indicate that 138-N and 180-N can assemble into a heterodimeric complex that contains both a truncated and an intact DHC and is predicted to contain a single motor domain. Other work [4,5] supports a similar role for the amino-terminus of the DHC in the assembly of the dimeric motor complex.

A characteristic feature of the dynein motor is its release from microtubules in the presence of ATP. Strikingly, we found that the single-headed dynein complex remains bound to microtubules in the presence of 5 mM MgATP

Figure 1

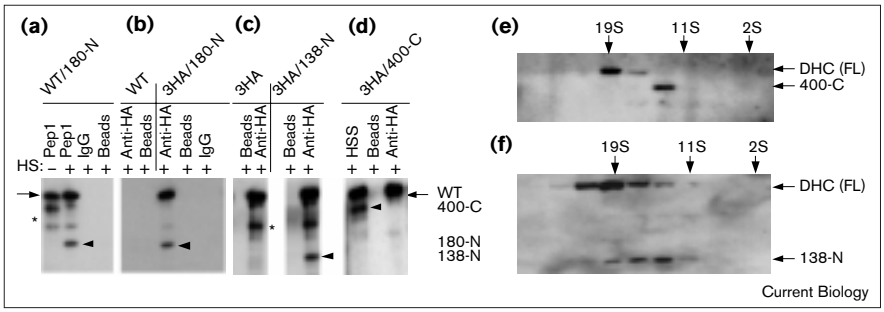


Summary of DHC transgenic products and sites of epitopes for anti-DHC antibodies. **(a)** The top line shows a schematic representation of *Drosophila* full-length DHC. The amino-terminal end is to the left and phosphate-binding P-loop motifs (P1–P4) are indicated by vertical dashed lines. The DHC designated 3HA is tagged with a triple copy of the hemagglutinin (HA) epitope at the site marked with an inverted triangle. Five additional DHC transgenic products were analyzed, indicated on the right. Predicted structures that bind microtubules are shown at the far right. **(b)** Schematic representation of the full-length DHC showing the approximate locations of epitopes recognized by the antibodies used in this study. See Supplementary material for details.

(Figure 3a; WT/138-N, lane TP). Under the same conditions, the native two-headed dynein complex showed little or no microtubule binding (Figure 3a; WT, lane TP). In the absence of the native homodimer, quantitative densitometry revealed a 1:1.1 stoichiometry between the 138-N fragment and the intact DHC, similar to the 1:1 ratio expected for the attachment of a single-headed heterodimeric complex. A similar stoichiometric association was observed with heterodimers containing 180-N. Because 138-N, 180-N and 380-N (data not shown) lacked the DHC motor domain, the attachment of the heterodimer was predicted to occur by the binding of the partner motor domain of the intact DHC. This would agree with observations made for heterodimeric single-headed kinesins [6]. Furthermore, the stoichiometry of binding supports the interpretation that binding is mediated by the motor domain of the intact DHC present in a single-headed heterodimer. If 138-N or 180-N exhibited a non-specific or spurious interaction with microtubules, then one would not expect to see 1:1 stoichiometry. The unexpected finding here is that the partner DHC motor domain remained attached to microtubules even in the presence of 5 mM MgATP.

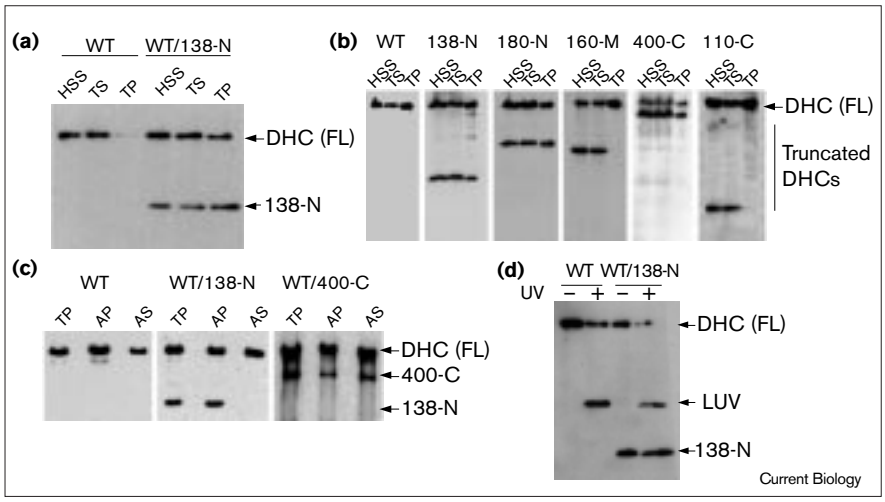
In the absence of ATP, native homodimeric complex, heterodimeric complexes that incorporate 138-N or 180-N, as well as the 400-C monomeric motor domain, all cosedimented with taxol-polymerized microtubules (Figure 3b,c; TP lanes). Cosedimentation depended on the presence of microtubules. In the absence of microtubules, after 10 hours on ice, all the DHC polypeptides remained soluble following centrifugation at 100,000 × *g* for 40 minutes. These results indicate that the DHC fragments did not pellet as a result of aggregation or precipitation from solution. The specificity of microtubule binding is also supported by the finding that a 160 kDa fragment (160-M) from the middle of the DHC, as well as a carboxy-terminal 110 kDa fragment (110-C) failed to partition with microtubules (Figure 3b; TP lanes). Furthermore, once bound to microtubules in the absence of MgATP, the two-headed dynein complex could be induced to detach from the microtubule lattice by the

Figure 2



Amino-terminal DHC fragments associate with the full-length (FL) DHC. **(a–d)** Immunoblot analysis of immunoprecipitation experiments. Heat-shock (HS) induction of the transgene is indicated by a +. DHC products were detected using appropriate anti-DHC antibodies. Beads, protein-G–agarose beads used as a control (see Supplementary material). IgG, immunoglobulin G. (a) In extracts from flies containing the 180-N transgene (WT/180-N) induced by heat shock, immunoprecipitation of the wild-type (WT) full-length DHC with the antibody Pep1 pulls down the 180-N fragment (lane + Pep1). In the absence of heat shock, only full-length DHC is precipitated (lane – Pep1). (b) In extracts from flies expressing 3HA and 180-N transgenes (3HA/180-N), anti-HA antibody co-immunoprecipitates the 3HA-tagged DHC and associated 180-N (lane + Anti-HA). The anti-HA antibody does not recognize the endogenous DHC in extracts from wild-type flies (WT, lane + Anti-HA). (c) The HA antibody precipitates the 138-N fragment in association with the 3HA-tagged DHC (3HA/138-N, lane + Anti-HA). Only the 3HA-tagged full-length DHC is pulled down by the HA antibody in precipitates from extracts lacking the 138-N product (3HA, lane + Anti-HA). (d) The HA antibody fails to precipitate the 400-C fragment in association with 3HA DHC (lane + Anti-HA). The 400-C fragment remains in the supernatant (lane + HSS). Asterisks mark apparent breakdown products of DHC. **(e,f)** Immunoblot analysis of sucrose density fractionation of high-speed supernatants from flies expressing (e) 400-C or (f) 138-N. Vertical arrows at the top indicate the positions of sedimentation standards.

Figure 3



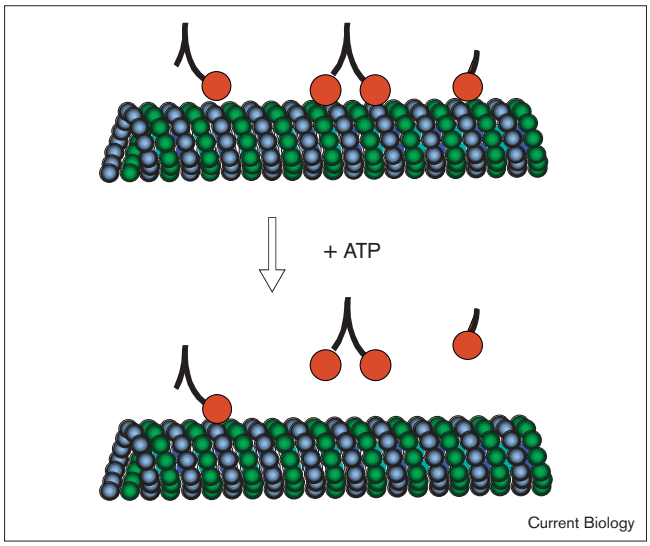
Single-headed heterodimeric dynein exhibits ATP-insensitive binding to microtubules. Appropriate anti-DHC antibodies were used in immunoblots. **(a)** Cosedimentation of DHC polypeptides in the presence of taxol-polymerized microtubules and 5 mM ATP. Microtubule binding of DHC polypeptides was analyzed in extracts from flies that expressed only wild-type full-length (FL) DHC (WT), or both the wild-type DHC and 138-N (WT/138-N). HSS, high-speed supernatant; TS, supernatant from taxol-treated extracts; TP, pellet from taxol-treated extracts. Equal amounts of protein for each fraction were analyzed by immunoblot analysis using the P_1H_4 antibody, which detects both full-length DHC and 138-N polypeptides. The native two-headed dynein complex does not cosediment with microtubules in the presence of 5 mM MgATP (WT, lane TP). Coexpression of 138-N and assembly of a single-headed dynein complex results in stoichiometric binding of the full-length DHC and 138-N to microtubules (WT/138-N, lane TP). **(b)** Partitioning of DHC polypeptides into microtubule pellets in the absence of ATP. The 138-N, 180-N and 400-C fragments partition into the microtubule pellet fraction (TP lanes). The 160-M and 110-C fragments do not partition into the microtubule pellet. **(c)** Microtubule pellets with associated DHC polypeptides (TP) were resuspended in 10 mM MgATP plus 20 μM taxol and re-pelleted to yield ATP supernatants (AS) and ATP-extracted microtubule pellets (AP). The full-length DHC and 400-C polypeptides are induced to dissociate from microtubules in the presence of ATP (all AS lanes). These DHC polypeptides are greatly enriched in the AS lanes, as the amount of AS sample loaded for the analysis is only one-tenth that of the AP sample loaded. None of the 138-N polypeptide is released from microtubules in the presence of ATP (WT/138-N lanes). **(d)** Immunoblot shows UV- and vanadate-mediated cleavage of wild-type dynein homodimeric complexes (WT) and heterodimeric dynein complexes (WT/138-N). The dynein samples were incubated in vanadate, in either the presence (+) or absence (–) of UV light. The DHC antibody P_1H_4 detects the ~200 kDa amino-terminal LUV cleavage product and 138-N, as well as the uncleaved DHC (FL).

addition of ATP (Figure 3c; WT, lane AS). Similarly, 400-C was also released from microtubules on addition of ATP (Figure 3c; WT/400-C). Significantly, when microtubules that had both two-headed and single-headed dynein complexes attached (WT/138-N) were resuspended in 10 mM MgATP, only the two-headed dynein complex detached from the microtubules, as only the intact heavy chain was detected in the ATP supernatant. The 138-N fragment and some of the intact heavy chain was not released (Figure 3c; WT/138-N; compare lanes AS and AP). Similar results were obtained with 180-N and 380-N (data not shown). These results are consistent with the single-headed heterodimer binding to microtubules in an ATP-insensitive fashion (Figure 3a).

Does the heterodimer retain ATP hydrolytic activity? To test this possibility, we asked whether the intact DHC of the heterodimer is a substrate for photocleavage with UV and vanadate. Previous work has shown that in the presence of UV light and vanadate, DHCs are cleaved near the hydrolytic nucleotide-binding site, P-loop1 (P1), to generate two DHC fragments [7]. Vanadate replaces the phosphate derived from ATP hydrolysis and mediates UV photolysis of the DHC, which results in the loss of ATPase activity [6]. To enrich for single-headed heterodimeric dynein complexes present in the extracts from *Drosophila* transgenic lines, we assembled microtubules in the presence of 5 mM MgATP. Under these conditions, the native homodimeric dynein complex did not bind to microtubules with high affinity (Figure 3a). Significantly, heterodimeric dynein did exhibit cleavage of the intact DHC within the complex (Figure 3d; WT/138-N, lane +). The 138-N fragment lacked the P1 domain and, as expected, was not cleaved. In parallel, native homodimeric dynein was purified by microtubule affinity in the absence of ATP from extracts that lacked truncated DHCs. As observed previously, the intact DHCs of the homodimer were cleaved in the presence of UV light and vanadate (Figure 3d; WT, lane +). These results show that the single motor domain within the attached heterodimer can still bind and hydrolyze ATP. Furthermore, the vanadate-mediated cleavage of the intact DHC demonstrates that an ADP–vanadate complex could form at the active site of the single motor domain. By these criteria, the single motor domain within the heterodimeric dynein complex retains normal ATP hydrolytic activity.

The wild-type dynein complex contains two DHCs and motor domains that interact cyclically with microtubules in the presence of MgATP. Our results show that the heterodimer fails to detach from microtubules in the presence of MgATP. We suggest that the failure of the heterodimer

Figure 4



Summary schematic diagram illustrating the microtubule-binding properties of dynein molecules. The behavior of heterodimeric (left), homodimeric (center), and monomeric (right) molecules (see Figure 1 and text) before and after addition of ATP are portrayed.

to detach in the presence of MgATP results from the loss of cooperative interactions with the partner motor domain. Although our results do not entirely exclude alternate explanations based on the properties of exposed domains within the truncated polypeptides, control experiments on the transgenic products support our interpretation. Kinetic analysis of the axonemal dynein ATPase has indicated that ATP binding induces rapid detachment of axonemal dynein from the microtubule [8]. Following ATP hydrolysis and phosphate release [9], the motor domain rebinds the microtubule. Force production is coupled to ADP release. Our data show that the microtubule–dynein heterodimer complex can bind ATP and hydrolyze ATP, but that the motor domain does not dissociate from the microtubule during this cycle of ATP turnover. These results are consistent with the hypothesis that the partner motor domain is required for ATP-induced detachment from the microtubule.

In summary, our work provides evidence for cooperative interactions between the two motor domains within the cytoplasmic dynein complex (Figure 4). Our results also reveal that the single monomeric dynein motor domain, 400-C, is capable of ATP-induced dissociation from the microtubule, suggesting that intermolecular interactions may be regulated in part through the stalk and dynein-associated polypeptides. In this regard, there is evidence that conformational transitions can be propagated through the tail of the dynein complex [10] and that mutations within the intermediate chain can influence the activity of the DHC [11].

The proposed cooperative interactions for cytoplasmic dynein support recent evidence that dimeric molecular motors of divergent origins use intermolecular interactions for unidirectional force generation and movement [12]. These cooperative interactions may lead to processivity, as observed in kinesin and the *Escherichia coli* Rep helicase. Yet motor domains may be cooperative and nonprocessive, like myosin II and *Drosophila* Ncd [13,14]. There is limited evidence that the cytoplasmic dynein motor is capable of processive movement [15]. It will be important to determine whether cooperative interactions between the motor domains of cytoplasmic dynein can be regulated to contribute to processive movement of the motor and its cargoes.

Supplementary material

Supplementary material including additional methodological details is available at http://current-biology.com/supmat/supmatin.htm.

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References

1. Vallee RB, Wall JS, Paschal BM, Shpetner HS: **Microtubule-associated protein 1C from brain is a two-headed cytosolic dynein.** *Nature* 1988, **332:**561-563.
2. Li M, McGrail M, Serr M, Hays TS: ***Drosophila* cytoplasmic dynein, a microtubule motor that is asymmetrically localized in the oocyte.** *J Cell Biol* 1994, **126:**1475-1494.
3. Koonce MP, Samsó M: **Overexpression of cytoplasmic dynein's globular head causes a collapse of the interphase microtubule network in *Dictyostelium*.** *Mol Biol Cell* 1996, **7:**935-948.
4. Koonce MP, Knecht DA: **Cytoplasmic dynein heavy chain is an essential gene product in *Dictyostelium*.** *Cell Motil Cytoskeleton* 1998, **39:**63-72.
5. Sakakibara H, Takada S, King SM, Witman GB, Kamiya R: **A *Chlamydomonas* outer arm dynein mutant with a truncated beta heavy chain.** *J Cell Biol* 1993, **122:**653-661.
6. Hancock WO, Howard J: **Processivity of the motor protein kinesin requires two heads.** *J Cell Biol* 1998, **140:**1395-1405.
7. Lee-Eiford A, Ow RA, Gibbons IR: **Specific cleavage of dynein heavy chains by ultraviolet irradiation in the presence of ATP and vanadate.** *J Biol Chem* 1986, **261:**2337-2342.
8. Porter ME, Johnson KA: **Transient state kinetic analysis of the ATP-induced dissociation of the dynein-microtubule complex.** *J Biol Chem* 1983, **258:**6582-6587.
9. Holzbaur EL, Johnson KA: **ADP release is rate limiting in steady-state turnover by the dynein adenosinetriphosphatase.** *Biochemistry* 1989, **28:**5577-5585.
10. Inaba K: **ATP-dependent conformational changes of dynein: evidence for changes in the interaction of dynein heavy chain with the intermediate chain 1.** *J Biochem* 1995, **117:**903-907.
11. Mitchell DR, Kang Y: **Reversion analysis of dynein intermediate chain function.** *J Cell Sci* 1993, **105:**1069-1078.
12. Lohman TM, Thorn K, Vale RD: **Staying on track: common features of DNA helicases and microtubule motors.** *Cell* 1998, **93:**9-12.
13. Conibear PB, Geeves MA: **Cooperativity between the two heads of rabbit skeletal muscle heavy meromyosin in binding to actin.** *Biophys J* 1998, **75:**926-937.
14. deCastro MJ, Ho CH, Stewart RJ: **Motility of dimeric ncd on a metal-chelating surfactant: evidence that ncd is not processive.** *Biochemistry* 1999, **38:**5076-5081.
15. Wang Z, Khan S, Sheetz MP: **Single cytoplasmic dynein molecule movements: characterization and comparison with kinesin.** *Biophys J* 1995, **69:**2011-2023.