Cytoplasmic dynein and dynactin in cell division and intracellular transport

Sher Karki* and Erika LF Holzbaur†

Since the initial discovery of cytoplasmic dynein, it has become apparent that this microtubule-based motor is involved in several cellular functions including cell division and intracellular transport. Another multisubunit complex, dynactin, may be required for most, if not all, cytoplasmic dynein-driven activities and may provide clues to dynein's functional diversity. Recent genetic and biochemical findings have illuminated the cellular roles of dynein and dynactin and provided insight into the functional mechanism of this complex motor.

Addresses
University of Pennsylvania, Department of Animal Biology, 143 Rosenthal Building, 3800 Spruce Street, Philadelphia, PA 19104-6046, USA
*e-mail: karkis@vet.upenn.edu
†e-mail: holzbaur@vet.upenn.edu

Current Opinion in Cell Biology 1999, **11**:45–53

http://biomednet.com/elecref/0955067401100045

© Elsevier Science Ltd ISSN 0955-0674

Abbreviations
cDHC1 cytoplasmic dynactin heavy chain 1
GFP green fluorescent protein
HAP1 Huntingtin-associated protein 1

Introduction

The first decade of research on the cytoplasmic form of dynein has produced some interesting surprises. Unlike the functional specialization that is the hallmark of many members of the kinesin superfamily [1], one major form of dynein appears to function in many different cellular roles. Although there is evidence that some of dynein's functional diversity is achieved through the use of alternate isoforms of dynein subunits, a single major form of dynein appears to drive very diverse cellular processes such as the transport of vesicles into the Golgi and the organization of the mitotic spindle. Also unexpected is the apparent requirement for dynactin as an activator or cofactor in most 'if not all' of these functions. As there have been a number of recent and comprehensive reviews on the molecular biology and biochemistry of dynein and dynactin [2,3] in this review we focus on key new findings which provide mechanistic insight into the adaptation of this single motor to many functions.

Axonemal dynein was first discovered as an ATPase that played an essential role in ciliary and flagellar beating [4]. A morphologically similar but distinct cytoplasmic form of dynein was subsequently discovered that could translocate organelles toward the minus ends of microtubules [5–7]. Cytoplasmic dynein is a multisubunit protein (1.2 MDa) consisting of two heavy chains of ~500 kDa each of which fold to form the two heads of the motor, as well as multiple intermediate chains (~70–74 kDa each), light intermediate chains (~53–59 kDa; [6,8]), and light chains (~8–22 kDa; [9,10]).

Dynactin — initially identified as a co-purifying set of polypeptides in dynein preparations from rat liver and testis [11] — is also a large multisubunit complex consisting of at least seven distinct polypeptides ranging in size from 22–150 kDa (reviewed in [3]). Dynactin was first demonstrated to be required for dynein-mediated transport *in vitro* [12], and is now believed to be required for most, or all, of dynein-mediated cellular activities. Genetic studies in *Saccharomyces cerevisiae*, *Neurospora crassa*, *Aspergillus nidulans*, and *Drosophila melanogaster* suggest that there is a genetic interaction between dynein and dynactin (reviewed in [3]); direct binding has been confirmed by biochemical analyses [13,14].

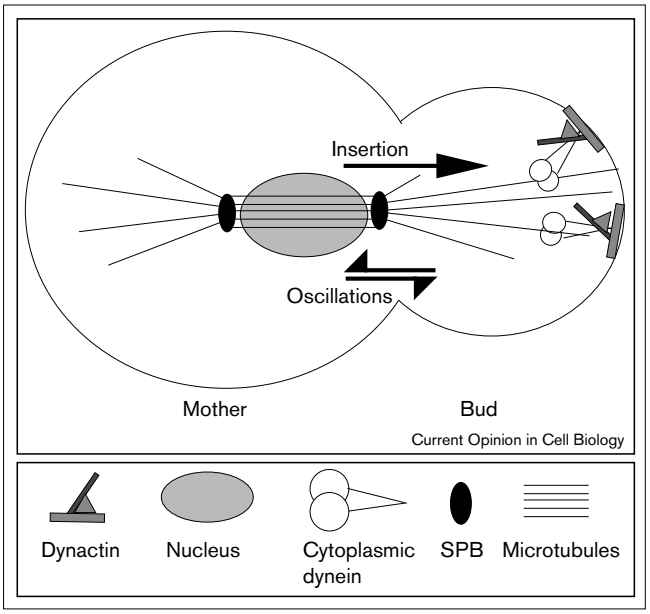
Unlike the extensive kinesin superfamily (reviewed in [1]), only a few isoforms of cytoplasmic dynein heavy chain family have been described to date [15–19]. Therefore, in order to carry out the diverse array of functions attributed to cytoplasmic dynein, the functional specialization as well as the temporal and spatial regulation of this motor might be governed by several mechanisms: these include isoform diversity of intermediate or light chains, the use of functional adaptors like dynactin, and post-translational modifications such as phosphorylation. Although we know little about how cytoplasmic dynein is specifically targeted to its presumptive sites of function or how its activity might be regulated, studies in the past few years have significantly clarified the role of dynein and dynactin in many subcellular activities — for example, the organization and orientation of the mitotic spindle, nuclear migration, vesicle trafficking, and retrograde axonal transport.

Spindle orientation and nuclear migration

Whereas initial knockout studies in *S. cerevisiae* clearly revealed a role for cytoplasmic dynein [20,21] and dynactin [22,23] in mitosis, deletion mutations of these proteins are not lethal. The replicative cycle apparently limps along in yeast in the absence of dynein or dynactin producing a significantly higher than normal proportion of binucleate or anucleate daughter cells. This 'partial' phenotype was initially confusing, but can now be understood in light of recent sophisticated studies from a number of laboratories. A deficiency in dynein is overcome, in part, by a kinesin-like motor, Kip3p, which also appears to participate in correctly orienting the mitotic spindle between mother and bud. Careful observations by DeZwaan *et al.* [24••], however, suggest that Kip3p and dynein have predominantly distinct roles in this process — Kip3p acts to correctly position the spindle near the mother–bud neck

before anaphase, and dynein is involved in the insertion of the spindle through the neck during anaphase (see Figure 1). Mitosis can proceed without one motor function or the other, but loss of both dynein and Kip3p in a double deletion strain is lethal. Surprisingly, however, the deletion of a third gene which encodes the kinesin-like protein Kip2p was found to suppress the lethality of the double mutant. This observation suggests that cell division in yeast is a balancing act among several motors.

Figure 1



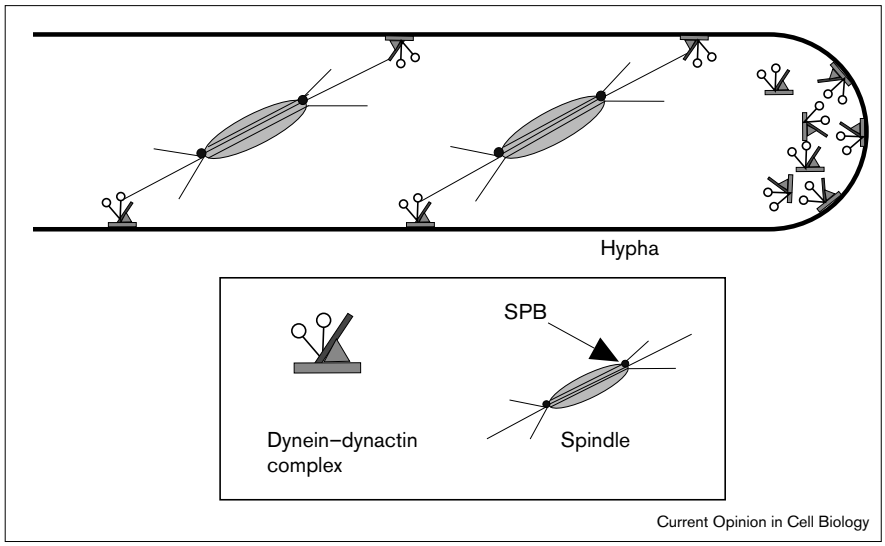
A model for the role of dynein–dynactin during anaphase nuclear migration in *Saccharomyces cerevisiae*. While the genes encoding subunits of dynein or dynactin are not essential in yeast, the dynein–dynactin complex has been shown to be involved in mitosis in a function that partially overlaps with the kinesin-related protein Kip3p. DeZwaan *et al.* [24••] propose that Kip3p and dynein are involved in distinct phases of nuclear migration: Kip3p functions to orient and position the nucleus near the bud neck before anaphase whereas cytoplasmic dynein is involved in inserting the nucleus through the bud neck during anaphase. DeZwaan *et al.* [24••] also propose that dynein is responsible for the oscillatory movements of the nucleus observed during nuclear insertion. This model is adapted from DeZwaan *et al.* [24••] and Holleran *et al.* [3].

Further details on the specific role of dynein in yeast emerged from studies using green fluorescent protein (GFP)-labeled tubulin to monitor microtubule dynamics in live cells. Yeast were demonstrated to undergo the type of microtubule dynamics that have been well-characterized in amphibian and mammalian cells (reviewed by Cassimeris in this issue [25]). Analysis of microtubule dynamics in a yeast strain lacking dynein revealed that the loss of this motor altered the parameters of the dynamic behavior: both microtubule growth and shrinkage rates were slowed in the absence of dynein. The frequency of catastrophe was reduced in the dynein-deleted strain, and many cells exhibited 'nondynamic' microtubules [26••]. The mechanism for this dynein-induced destabilization of microtubules remains to be determined, but may resemble the Kar3p destabilization of microtubule minus ends [27]. Further studies by Shaw *et al.* [28•] have emphasized the key role of astral microtubule dynamics in the orientation of the mitotic spindle. Astral microtubules were observed to undergo a searching process followed by transient interactions between microtubules and the bud cortex. These interactions between the microtubule and the bud cortex may be mediated by dynein and dynactin [28•]. The proposed model suggests that the dynein–dynactin complex may interact directly or indirectly with the polarized actin cytoskeleton of yeast.

Whereas the results of DeZwaan *et al.* [24••] suggest that, in yeast, dynein does not move the nucleus to the mother–bud neck, both dynein and dynactin have been found to be required for nuclear migration in filamentous fungi. In both *Aspergillus nidulans* and *Neurospora crassa*, mutations in genes encoding dynein or dynactin subunits result in nuclear clumping, and a significant inhibition of the migration of nuclei out along the hyphae, the elongating germ tubes of these filamentous fungi (reviewed in [3]). Although immunocytochemical studies indicate that dynein is located at the hyphal tip [29], a very plausible model for the process of nuclear migration recently proposed by Efimov and Morris [30••] suggests that it is dynein bound at sites along the cell cortex of the hyphae that exerts force on astral microtubules radiating outward from spindle pole bodies. The pulling force generated by the cortical dynein would then result in the spacing out of nuclei along the hyphae (see Figure 2). This model leaves the question of how dynein is localized to cortical sites along the hyphae unanswered, but, as with the consensus yeast model discussed above, the most likely explanation is an association between dynactin and the cortical actin cytoskeleton.

Several laboratories have taken advantage of the fact that dynein is not required for cell viability in either yeast or fungi, and have constructed screens for genes which are synthetically lethal with dynein mutations — meaning that in the absence of dynein these genes are essential. As described above, the kinesin-like motor Kip3p was found to be synthetically lethal with dynein, in that cells lacking both these proteins cannot divide [24••,31•]. In yeast, the deletion of another kinesin-like motor, Cin8p, is lethal in a strain lacking dynein [32•]. In *Aspergillus*, six genes are essential in the absence of either dynein or the Cin8p homologue, bimC [30••]. Two of these genes have been cloned and identified as homologues of the yeast spindle assembly checkpoint genes *BUB1* and *BUB3*. This observation is of particular interest given the recent work identifying dynein's role in spindle assembly in higher eukaryotes (summarized below). In yeast, a synthetic lethality screen also led to the identification of proposed checkpoint genes. Muhua *et al.* [33•] examined the delay in cytokinesis that occurs in yeast when the spindle is misaligned as a result of a dynactin mutation, and found that

Figure 2



A model for the role of dynein and dynactin in nuclear migration in *Aspergillus nidulans*. A notable feature of filamentous fungi is the even distribution of nuclei along the fungal hypha. A number of studies have demonstrated that this process requires the activities of dynein and dynactin (reviewed in [3]). Efimov and Morris [30••] have recently suggested a model describing a mechanism that would allow the even distribution and proper positioning of nuclei while the hyphal tip is growing. According to this model, cytoplasmic dynein – which is presumably anchored to the cell cortex through dynactin – is located at sites where the nuclei need to be positioned. Although it is not yet clear how dynein may be specifically localized along the hyphae, this distribution of dynein would ensure the correct positioning of daughter nuclei. Dynein is known to localize to the tip of the hyphae, presumably in association with the cortex. This model is adapted from Efimov and Morris [30••]. SPB, spindle pole body.

this delay was over-ridden by a mutation in a gene encoding the yeast homologue of EB1[91]. EB1 is a human protein which has been shown to bind to the tumor suppressor protein APC [91] and associate with cellular microtubules ([92], and references therein). Muhua *et al.* [33•] hypothesize that EB1 is part of a checkpoint control mechanism that acts just before cytokinesis and functions to prevent aneuploidy under conditions in which the mitotic spindle becomes misaligned, perhaps due to the depolymerization of microtubules in a cold environment. Alternatively, if dynein and dynactin have a direct role in cytokinesis [34•], the analysis of this interaction may be more complex.

Spindle assembly

In higher eukaryotes, data from cellular and *in vitro* assays have clearly identified a role for dynein and dynactin in the assembly of the mitotic spindle. In initial studies, the microinjection of anti-dynein antibodies was shown to cause the disruption of mitotic spindle assembly in cultured mammalian cells ([35]; also see [36]). Disruption of the dynein–dynactin complex by overexpression of the dynamitin subunit of dynactin was also shown to disrupt the spindles of dividing cells [37]. *In vitro* studies using *Xenopus* oocyte extracts [38,39] or HeLa cell extracts [36,40] have provided insight into the process of spindle assembly — dynein and dynactin are required to focus the minus ends of microtubules at the spindle poles. Merdes *et al.* [38] give evidence for a stable association of dynein and dynactin with NuMA, a protein which was previously shown to be required for spindle assembly. The mechanism by which dynein and dynactin act to organize or focus the spindle microtubules remains to be determined but dynein–dynactin could presumably mediate the sliding of spindle microtubules relative to each other. As each of the heads of dynein can bind to microtubules — as can dynactin [41], and NuMA [38] — a mechanism involving the cross-linking and sliding of adjacent microtubules is clearly plausible.

The observation that the kinesin-like motor Eg5 may also be required for correct spindle assembly [38,42,43] further complicates the picture. The motor activity of Eg5, which is directed toward the plus ends of microtubules, appears to oppose the minus-end-directed motor cytoplasmic dynein. This observation is of particular interest because it has recently been demonstrated in yeast two-hybrid screens that the human homologue of Eg5 binds to the p150^{Glued} subunit of dynactin [44], which was previously shown to bind directly to dynein [13,14]. Although we are not yet at a stage where we can be confident we understand the mechanisms involved in mitotic spindle orientation or assembly, it is encouraging to observe that in both yeast and higher eukaryotes the balancing of oppositely-directed forces appears to be a key piece of the puzzle.

Dynein and dynactin at the kinetochore: anaphase in higher eukaryotes

While many studies have clearly shown that cytoplasmic dynein and dynactin are located at the kinetochore [34•,37,45,46,47••], the functional basis for this localization of the motor has been more difficult to assess. The observations of Vaisberg *et al.* [35] of a collapsed spindle upon microinjection of anti-dynein antibodies, and of Echeverri *et al.* [37] of a disrupted spindle upon overexpression of a dynactin subunit might both be explained by dynein's proposed role in the assembly of the mitotic spindle, described above. The recent observation that dynactin binds specifically to ZW10, a conserved and essential component of the kinetochore, however, may now allow more specific insight into the role of kinetochore-bound dynein. Starr *et al.* [47••]

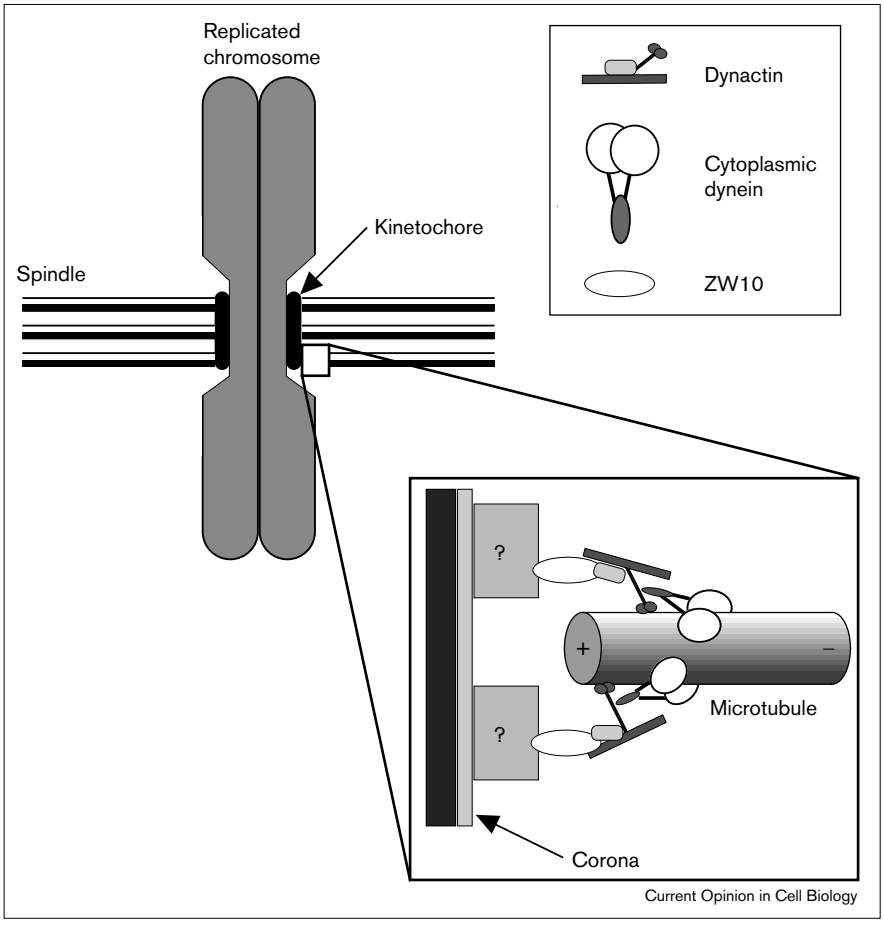
observed a direct interaction between ZW10 and the dynamitin subunit of dynactin in a two-hybrid screen. They also found that in flies carrying mutations in ZW10, dynein was no longer localized to the kinetochores (see Figure 3). Although the observed correlation between the loss of dynein–dynactin–ZW10 from the kinetochore and the phenotypic defects observed in chromosome segregation during mitosis in cells with ZW10 mutations does not prove a role for dynein in these processes, it raises interesting questions. Careful analysis of the kinetochore localization of these proteins throughout prophase, metaphase, and anaphase has suggested that the dynein–dynactin–ZW10 complex may be involved in a tension-sensitive checkpoint mechanism which acts to delay anaphase until all chromosomes have bipolar attachments, thus ensuring proper chromosomal segregation during anaphase [47••]. Alternatively, dynein may be required for the proper movement of chromosomes to the poles in anaphase. The latter role would be consistent with observations of the role of dynein and dynactin in anaphase in yeast [48,49].

The final stages of cell division: an additional role for dynein and dynactin?

Two recent studies have now localized cytoplasmic dynein to the developing cleavage furrow [34•,50•,51]. Dynactin was also localized to the furrow during telophase, and later to the midbody, the constricted remnant of the cleavage furrow which contains tightly packed microtubules surrounded by cell membrane [34•]. In some images, dynactin appears to be specifically localized to the cortex surrounding the tightly packed microtubules of the intracellular bridge [34•]. It is tempting to speculate that dynein and dynactin may mediate communication between the mitotic spindle and the actin cortex, participating in the correct localization of the cleavage furrow at the metaphase plate [52,53,54]. As dynactin can bind directly to microtubules and can also associate with actin either directly [55], or indirectly via actin-binding proteins [56], it is possible that dynactin may play a critical role in this process. Potentially, the dynein–dynactin complex might function actively as a motor in cytokinesis; recent studies on *Drosophila* mutants defective in cytokinesis have provided clear evidence that other microtubule-based motors [57–59] are involved in the mechanics of late stage cell division.

It is also intriguing to note that Campbell *et al.* [50•] have recently reported an *in vitro* binding interaction between the dynein light chain Tctex-1 and the Src-related tryosine kinase $p59^{fyn}$. Although these two proteins were differentially localized throughout most of the cell cycle, they

Figure 3



A model for the role of dynein and dynactin at the kinetochore. Both cytoplasmic dynein and components of dynactin have been localized to the kinetochore. Although many of the interactions involved have yet to be identified (indicated by a question mark in the figure), a critical interaction between the p50 (dynamitin) subunit of dynactin and ZW10, a conserved and essential component of the kinetochore, was recently demonstrated by Starr *et al.* [47••]. They propose that a complex containing ZW10 and other components which associates with the kinetochore corona binds to dynactin via the interaction between ZW10 and p50. The interaction between dynactin and dynein recruits the motor to the kinetochore. Both dynein and dynactin can bind to microtubules, and therefore may facilitate the capture of the free plus-ends of spindle microtubules. Dynein's ability to 'walk' toward the minus ends of microtubules may contribute to the separation of sister chromatids. This model is adapted from Starr *et al.* [47••].

showed a strikingly coincident co-localization at the cleavage furrow during cytokinesis. This observation of a transient association between dynein and a regulatory kinase may be a first step in more clearly establishing the spatial and temporal regulation of dynein within the cell.

Golgi dynamics

As the numerous studies summarized above indicate, dynein is involved in one or more steps in mitosis in higher eukaryotes, suggesting that a null mutation would be lethal. Harada *et al.* [60••] produced mice lacking dynein heavy chain 1 (DHC1) by gene targeting, and found no survival of embryos past 8.5 days postcoitum. Harada and coworkers, however, went on to analyze DHC1$^{-/-}$ blastocysts in culture and found that the Golgi complex in these cells was clearly disrupted, with fragments dispersed throughout the cytoplasm.

This observation was completely consistent with the elegant studies of Presley *et al.* [61••], in which Golgi dynamics were monitored in live cells using GFP-labeled vesicular stomatis virus glycoprotein as cargo. Dynein–dynactin function was perturbed by overexpressing the dynamitin subunit of dynactin, and this led to a significant block in the transport of pre-Golgi carrier structures from the endoplasmic reticulum to the Golgi. Holleran *et al.* [56] and Burkhardt *et al.* [62•] also observed the perturbation of Golgi structure upon overexpression of dynactin subunits.

The targeting of dynein to the Golgi remains to be clarified, but the studies of Holleran *et al.* [56] have revealed an interaction between dynactin and spectrin, which may mediate the attachment of the dynein–dynactin motor complex with the surface of organelles undergoing transport to the Golgi (reviewed in [63]). This interaction is presumably mediated by the Arp1 subunit of dynactin (E Holleran, MC Stanekwich, J Morrow, E Holzbaur, unpublished data), which forms an actin-like filament at the base of the dynactin complex [55,64••]. It is interesting to contrast this mechanism to the recently described role of the p50 subunit of dynactin in linking dynein to ZW10 at the kinetochore. In both cases, dynactin may serve as a binding platform, but dynactin itself may be differentially localized in the cell via specific interactions mediated by different subunits. Further investigations of this hypothesis are clearly warranted.

Neuronal transport

Cytoplasmic dynein was originally purified from neuronal tissue and was first characterized as a retrograde microtubule-based motor [65,66]. More recent studies have examined dynactin's role in neuronal transport, as well as the possibility that dynein and dynactin may be involved in the pathogenesis of neuronal disorders. Waterman-Storer *et al.* [67••] demonstrated that an antibody to the p150^{Glued} subunit of dynactin that blocked the binding of dynein to dynactin also inhibited the bi-directional motility of vesicles along microtubules in extruded squid axoplasm. This result showed that the dynein–dynactin interaction is functionally significant in fast axonal transport. Waterman-Storer *et al.* (67••) also demonstrated that in the presence of the antibody dynein was blocked from its association with vesicles. This observation is further evidence for the model which suggests that dynactin acts as a binding platform for dynein.

In addition to a role in fast axonal transport, dynein–dynactin function is also likely to be important in the growth, development, and organization of neurites. Ahmad *et al.* [68••] microinjected the 50 kDa subunit of dynactin (dynamitin) into mature neurons that had been depleted of microtubules. They noted that, in contrast to control cells, microtubules failed to translocate into the axon; furthermore, axonal outgrowth was inhibited by the presence of excess p50 [68••].

The initial observation that the 150 kDa subunit of vertebrate dynactin was homologous to the product of the *Drosophila* gene *Glued* suggested an essential role for dynein and dynactin in neuronal development [69,70] as mutations in the *Glued* gene caused developmental defects in the *Drosophila* compound eye and in the neurons of the optic lobe [71]. Mutations in cytoplasmic dynein subunits were subsequently shown to result in a similar rough eye phenotype [72]. More recent, detailed studies on compound eye development in flies carrying a mutation in Glued have found that the product of this gene is involved in multiple processes during development including mitosis, nuclear migration, cell fate determination, rhabdomere morphogenesis, and cell death [73•]. In a separate study, the Gl^{1} mutation was found to disrupt sensory path finding during metamorphosis [74•]. Axonal growth rates did not appear to be affected, and pathfinding was not disrupted at a gross level, as axons did reach the appropriate domains; however, axonal branching within a domain was disrupted in both the strain with Glued mutations [74•] and in a mutant strain lacking a cytoplasmic dynein light chain [75]. Disruption of axonal branching in the strain with Glued mutations is apparently correlated with a disrupted reflex response, suggesting that disruption of dynein and dynactin has an effect on either synaptic connectivity or synaptic transmission [74•].

These observations of neuronal disruptions linked to mutations in either dynein or dynactin in *Drosophila* suggest that there might be an involvement of dynein and dynactin in human neuropathologies. Recent studies have identified a potential link between dynactin and Huntington's disease (HD), a progressive neurodegenerative condition in adults. This disease has been shown to be caused by the expansion of a polyglutamine tract in the Huntingtin gene [76]. The product of this gene, huntingtin, associates with a brain-specific protein, Huntingtin-associated protein 1 (HAP1), in a glutamine repeat length-dependent manner suggesting that this interaction may be pathologically relevant [77].

Using the yeast two-hybrid system, affinity chromatography and immunoprecipitations, Engelender *et al.* [78] and Li *et al.* [79] have demonstrated that HAP1 also binds to the $p150^{Glued}$ subunit of dynactin. The observed interaction suggests that dynein–dynactin function may be essential for the normal functioning, processing, or transport of HAP1 and potentially of the huntingtin polypeptide.

Possible links between dynein–dynactin and other neurodegenerative diseases such as limb-girdle muscular dystrophy type 2 B [80], and Alzheimer's disease [81] remain to be explored more fully. Perhaps better understood at present is the role of cytoplasmic dynein in mediating the infection and spread of viral pathogens. Sodeik *et al.* [82•] looked at herpes simplex virus 1 transport in cultured mammalian cells, and found that the transport of the viral capsid to the nucleus was dependent on intact microtubules. Cytoplasmic dynein was found in proximity to the viral capsids being actively transported to the nucleus [82•]. The dynein-mediated retrograde transport of viral capsids from the site of fusion at the presynaptic membrane along the axon to the nucleus may be a critical part of viral infections of the nervous system, and again warrants further research.

Conclusion

Dynein was first identified as a molecular motor in the 1960s, but significant questions about the mechanism of dynein motor function remain. The function of multiple nucleotide binding sites per heavy chain [83,84], and the nature of the microtubule-binding domain [85••,86] remain to be resolved. Perhaps one of the key mechanistic questions is whether dynactin is always required to 'activate' dynein function and, if so, why? Genetic, functional, and biochemical studies to date all suggest that dynactin is required for all cellular functions in which cytoplasmic dynein is involved (reviewed in [3]); however, dynein alone is sufficient to drive microtubule gliding *in vitro*. Dynein and dynactin can interact both *in vitro* and *in vivo*, but in the cell, a large proportion of each of the two complexes do not appear to be tightly associated.

Although it remains possible that dynactin may activate dynein through an enzymatic or allosteric interaction, accumulating data suggest that dynactin actually functions as a binding platform which mediates the association of dynein with its cargo. For example, Waterman–Storer *et al.* [67••] demonstrated that disruption of the dynein–dynactin interaction disrupts the association of dynein with vesicles, and therefore blocks the transport of these vesicles along microtubules. The striking composition and morphology of dynactin favors this hypothesis, as the most notable feature of the complex is a filament formed from the actin-related protein Arp1 [55,64••]. This Arp1 filament resembles the short actin filaments found in the erythrocyte cell membrane. Perhaps not surprisingly Holleran *et al.* [56] have detected an interaction between dynactin and spectrin, which is presumably mediated by Arp1 and thus may be analogous to the actin–spectrin interaction in the red cell membrane. An association between dynactin and spectrin may target dynactin to intracellular vesicles; specific associations between Arp1 and other cellular proteins may target dynein and dynactin to other intracellular sites of function. A similar mechanism has been proposed for the interaction of dynein–dynactin with the cell cortex which is a critical feature of the models for dynein function in spindle orientation and nuclear migration discussed above. In contrast, it appears that while dynactin may also mediate the association of dynein with the kinetochore, in this case the targeting is due to a direct interaction between the dynamitin subunit of dynactin and the ZW10 component of the kinetochore. It is interesting to note that dynamitin, found at a stoichiometry of ~5 per mole of dynactin [87], is also thought to self-associate. As with centractin, this self-association may allow each molecule of dynactin to make multiple binding contacts with the cargo to be transported.

Given the multiple microtubule-binding sites within the dynein–dynactin complex, it is also easy to postulate that the active motor complex may mediate microtubule cross-linking or the sliding of adjacent microtubules, consistent with a role in spindle assembly (see [3]). Alternatively, dynein's role in spindle assembly may hinge on its ability to concentrate its cargo NuMA at the minus ends of spindle, in which case this role would be more analogous to its role in vesicle transport. It has also been hypothesized that the role of the additional microtubule-binding sites found on the $p150^{Glued}$ subunit of dynactin may be to make the motor complex more processive; that is, to ensure that dynein will transport its cargo a significant distance along the microtubule per binding encounter [41]. This is currently an active area of research.

Future directions

At present, a major gap in our knowledge is the mechanisms that allow temporal and spatial control of the dynein motor complex within the cell. Regulation would appear to be critical, given the numerous cellular functions attributed to this motor. Progress has been slow, but recent approaches offer promise, such as the identification of genes that interact with dynein, for example extragenic suppressors, or genes that are synthetically lethal with dynein mutations. Other approaches have focused on analyzing a potential role for phosphorylation in regulating dynein and dynactin [50•,88–90]. The issue of whether cytoplasmic dynein is processive in contrast to its axonemal counterpart and if not, whether the dynactin complex confers such property is central to understanding the seemingly obligatory role of dynactin in cytoplasmic dynein-based activities. Another question which needs to be addressed is the nature of the putative link between dynactin and the actin-based cytoskeleton, which may allow the specific targeting of dynein and dynactin to cortical sites of function. Describing the molecular mechanisms that allow the careful control of a single

motor, which drives both vesicle trafficking and mitotic dynamics, poses a significant challenge to stimulate further research in this area.

Acknowledgements

The authors gratefully acknowledge the critical insights of Tim Yen, David Roof, Kerry Campbell, and Frederick Holzbaur.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hirokawa N: **Kinesin and dynein superfamily proteins and the mechanism of organelle transport.** *Science* 1998, **279**:519-526.
2. Vallee RB, Gee MA: **Make room for dynein.** *Trends Cell Biol* 1998, **8**:490-494.
3. Holleran EA, Karki S, Holzbaur ELF: **The role of dynactin complex in intracellular motility.** *Int Rev Cytol* 1998, **182**:69-109.
4. Gibbons IR, Rowe A: **Dynein: a protein with adenosinetriphosphatase activity from cilia.** *Science* 1965, **149**:423.
5. Lye RJ, Porter ME, Scholey JM, McIntosh JR: **Identification of a microtubule-based cytoplasmic motor in the nematode *C. elegans*.** *Cell* 1987, **51**:309-318.
6. Paschal BM, Shpetner HS, Vallee RB: **MAP 1C is a microtubule-activated ATPase which translocates microtubules *in vitro* and has dynein-like properties.** *J Cell Biol* 1987, **105**:1273-1282.
7. Paschal BM, Vallee RB: **Retrograde transport by the microtubule associated protein MAP-1C.** *Nature* 1987, **330**:181-183.
8. Hughes SM, Vaughan KT, Herskovits JS, Vallee RB: **Molecular analysis of a cytoplasmic dynein light intermediate chain reveals homology to a family of ATPases.** *J Cell Sci* 1995, **108**:17-24.
9. King SM, Dillman JF III, Benashski SE, Lye RJ, Patel-King RS, Pfister KK: **The mouse *t*-complex-encoded protein Tctex-1 is a light chain of brain cytoplasmic dynein.** *J Biol Chem* 1996, **271**:32281-32287.
10. King SM, Barbarese E, Dillman JF III, Patel-King RS, Carson JH, Pfister KK: **Brain cytoplasmic and flagellar outer arm dyneins share a highly consereved M_r 8,000 light chain.** *J Biol Chem* 1996, **271**:19358-19366.
11. Collins CA, Vallee RB: **Preparation of microtubules from rat liver and testis: cytoplasmic dynein is a major microtubule-associated protein.** *Cell Motil Cytoskeleton* 1989, **14**:491-500.
12. Gill SR, Schroer TA, Szilak I, Steuer ER, Sheetz MP, Cleveland DW: **Dynactin, a conserved, ubiquitously expressed component of an activator of vesicle motility mediated by cytoplasmic dynein.** *J Cell Biol* 1991, **115**:1639-1650.
13. Karki S, Holzbaur ELF: **Affinity chromatography demonstrates a direct binding between cytoplasmic dynein and the dynactin complex.** *J Biol Chem* 1995, **270**:28806-28811.
14. Vaughan KT, Vallee RB: **Cytoplasmic dynein binds dynactin through a direct interaction between the intermediate chains and $p150^{Glued}$.** *J Cell Biol* 1995, **131**:1507-1516.
15. Gibbons BH, Asai DJ, Tang WJ, Hays TS, Gibbons IR: **Phylogeny and expression of axonemal and cytoplasmic dynein genes in sea urchins.** *Mol Biol Cell* 1994, **5**:57-70.
16. Tanaka Y, Zhang Z, Hirokawa N: **Identification and molecular evolution of new dynein-like protein sequences in rat brain.** *J Cell Sci* 1995, **108**:1883-1893.
17. Criswell PS, Ostrowski LE, Asai DJ: **A novel cytoplasmic dynein heavy chain: expression of DHC1b in mammalian epithelial cells.** *J Cell Sci* 1996, **109**:1891-1898.
18. Vaisberg EA, Grissom PM, McIntosh JR: **Mammalian cells express three distinct dynein heavy chains that are localized to different cytoplasmic organelles.** *J Cell Biol* 1996, **133**:831-842.
19. Criswell PS, Asai DJ: **Evidence for four cytoplasmic dynein heavy chain isoforms in rat testis.** *Mol Biol Cell* 1998, **9**:237-247.
20. Eshel D, Urrestarazu LA, Vissers S, Jauniaux JC, van Vliet-Reedijk JC, Planta RJ, Gibbons IR: **Cytoplasmic dynein is required for the normal nuclear segregation in yeast.** *Proc Natl Acad Sci USA* 1993, **90**:11172-11176.
21. Li Y-Y, Yeh E, Hays T, Bloom K: **Disruption of mitotic spindle orientation in a yeast dynein mutant.** *Proc Natl Acad Sci USA* 1993, **90**:10096-10100.
22. Clark SW, Meyer DI: **ACT3: a putative centractin homologue in *S. cerevisiae* is required for proper orientation of the mitotic spindle.** *J Cell Biol* 1994, **127**:129-138.
23. Muhua L, Karpova TS, Cooper JA: **A yeast actin-related protein homologous to that in vertebrate dynactin complex is important for spindle orientation and nuclear migration.** *Cell* 1994, **78**:669-679.
24. •• DeZwaan TD, Ellingson E, Pellman D, Roof DM: **Kinesin-related *KIP3P* of *Saccharomyces cerevisiae* is required for a distinct step in nuclear migration.** *J Cell Biol* 1997, **138**:1023-1040.

This report carefully examines the phenotypic effects of Kip3p and DHC1 mutations in *S. cerevisiae*. The authors conclude that in contrast to previous conclusions, dynein is not required for the initial movement of the nucleus to the mother–bud neck, but is required for translocation through the bud neck during anaphase.

25. Cassimeris L: **Accessory protein regulation of microtubule dynamics throughout the cell cycle.** *Curr Opin Cell Biol* 1999, **11**:134-141.
26. •• Carminati JL, Stearns T: **Microtubules orient the mitotic spindle in yeast through dynein-dependent interactions with the cell cortex.** *J Cell Biol* 1997, **138**:629-661.

The authors used green fluorescent protein labeled tubulin to monitor microtubule dynamics in live yeast cells. Microtubule dynamics were also analyzed in cells lacking dynein, and were found to differ significantly from those in wild-type cells. Perturbations in the interaction of microtubules with the cortex and misaligned spindles were observed in cells with dynein muations.

27. Endow SA, Kang SJ, Satterwhite LL, Rose MD, Skeen VP, Salmon ED: **Yeast Kar3 is a minus-end microtubule motor protein that destabilizes microtubules preferentially at the minus ends.** *EMBO J* 1994, **13**:2708-2713.
28. • Shaw SL, Yeh E, Maddox P, Salmon ED, Bloom K: **Astral microtubule dynamics in yeast: a microtubule-based searching mechanism for spindle orientation and nuclear migration into the bud.** *J Cell Biol* 1997, **139**:985-994.

This report describes the dynamics of astral microtubules in yeast cells expressing green fluorescent protein labeled dynein. They note that the hyperelongated and stable microtubules observed in cells expressing no dynein – or excess dynein – block nuclear migration into the bud, and conclude that nuclear migration into the bud involves a dynamic microtubule-based searching mechanism requiring dynein.

29. Xiang X, Roghi C, Morris NR: **Characterization and localization of the cytoplasmic dynein heavy chain in *Aspergillus nidulans*.** *Proc Natl Acad Sci USA* 1995, **92**:9890-9894.
30. •• Efimov VP, Morris NR: **A screen for dynein synthetic lethals in *Aspergillus nidulans* identifies spindle assembly checkpoint genes and other genes involved in mitosis.** *Genetics* 1998, **8**:1013-1018.

The authors identified nine genes in *Aspergillus nidulans* which are essential in the absence of dynein. Sequencing of two of these genes revealed homology with yeast spindle assembly checkpoint genes *BUB1* and *BUB3*. These observations suggest that dynein is involved in both mitosis and nuclear distribution in *Aspergillus*.

31. • Cottingham FR, Hoyt MA: **Mitotic spindle positioning in *Saccharomyces cerevisiae* is accomplished by antagonistically acting microtubule motor proteins.** *J Cell Biol* 1997, **138**:1041-1053.

The authors demonstrate partially overlapping functions for dynein and the kinesin-related protein Kip3p in the positioning of the spindle at the mother–bud neck during cell division in *Saccharomyces cerevisiae*. The functions are opposed by the kinesin-related protein Kip2p, as the deletion of *KIP2* suppresses the inviability seen in *dyn1Δkar3Δ*cells.

32. • Geiser JR, Schott EJ, Kingsbury TJ, Cole NB, Totis LJ, Bhattacharyya G, He L, Hoyt MA: ***Saccharomyces cerevisiae* genes required in the absence of the CIN8-encoded spindle motor act in functionally diverse mitotic pathways.** *Mol Biol Cell* 1997, **8**:1035-1050.

The authors screened for *Saccharomyces cerevisiae* genes which are synthetically lethal in the absence of Cin8p. Mutant alleles of eight genes were identified as causing phenoptypes similar to mutations in the dynein heavy chain gene. Several of these genes showed homology to previously characterized

dynein and dynactin genes, including the cytoplasmic dynein intermediate chain, and the p150^{Glued} and Arp1 subunits of dynactin.

33. Muhua L, Adames NR, Murphy MD, Shields CR, Cooper JA: **A**
• **cytokinesis checkpoint requiring the yeast homologue of an APC-binding protein.** *Nature* 1998, **393**:487-491.
The authors screened for mutations which override the delay in cytokinesis observed in yeast cells with a mutation in the Arp1 gene *Act5*. Two genes were identified and characterized, one was in a yeast homologue of EB1, a mammalian protein which has been shown to interact with the tumor suppressor protein APC. The second mutation was in a gene encoding a phosphatidylinositol-4-OH kinase. The authors propose that these genes may be part of a novel cytokinesis checkpoint mechanism.

34. Karki S, LaMonte B, Holzbaur ELF: **Characterization of the p22**
• **subunit of dynactin reveals the localization of cytoplasmic dynein and dynactin to the midbody of dividing cells.** *J Cell Biol* 1998, **142**:1023-1034.
During their characterization of the smallest dynactin subunit, p22, the authors noted a novel localization for cytoplasmic dynein and dynactin at the cleavage furrow and the midbody of dividing cells (also see [50•]). The authors also note the persistence of dynein's localization to the kinetochore post-metaphase.

35. Vaisberg EA, Koonce MP, McIntosh JR: **Cytoplasmic dynein plays a role in mammalian mitotic spindle formation.** *J Cell Biol* 1993, **123**:849-858.

36. Gaglio T, Dionne MA, Compton DA: **Mitotic spindle poles are organized by structural and motor proteins in addition to centrosomes.** *J Cell Biol* 1997, **138**:1055-1066.

37. Echeverri CJ, Paschal BM, Vaughan KT, Vallee RB: **Molecular characterization of 50 kD subunit of dynactin reveals function for the complex in chromosome alignment and spindle organization during mitosis.** *J Cell Biol* 1996, **132**:617-633.

38. Merdes A, Ramyar K, Vechio JD, Cleveland DW: **A complex of NuMA and cytoplasmic dynein is essential for mitotic spindle assembly.** *Cell* 1996, **87**:447-458.

39. Heald R, Tournebize R, Habermann A, Karsenti E, Hyman A: **Spindle assembly in *Xenopus* egg extracts: respective roles of centrosomes and microtubule self-organization.** *J Cell Biol* 1997, **138**:615-628.

40. Gaglio T, Saredi A, Bingham JB, Hasbani MJ, Gill SR, Schroer TA, Compton DA: **Opposing motor activities are required for the organization of the mammalian mitotic spindle pole.** *J Cell Biol* 1996, **135**:399-414.

41. Waterman-Storer CM, Karki S, Holzbaur ELF: **The p150^{Glued} component of the dynactin complex binds to both microtubules and the actin-related protein centractin (Arp-1).** *Proc Natl Acad Sci USA* 1995, **92**:1634-1638.

42. Sawin KE, LeGuellec K, Philippe M, Mitchison TJ: **Mitotic spindle organization by a plus-end-directed microtubule motor.** *Nature* 1992, **359**:540-543.

43. Sawin KE, Mitchison TJ: **Mutations in the kinesin-like protein Eg5 disrupting localization to the mitotic spindle.** *Proc Natl Acad Sci USA* 1995, **92**:4289-4293.

44. Blangy A, Arnaud L, Nigg EA: **Phosporylation by p34^{cdc2} protein kinase regulates binding of the kinesin-related motor HsEg5 to the dynactin subunit p150^{Glued}.** *J Biol Chem* 1997, **272**:19418-19424.

45. Pfarr CM, Coue M, Grissom PM, Hays TS, Porter ME, McIntosh JR: **Cytoplasmic dynein is localized to kinetochores during mitosis.** *Nature* 1990, **345**:263-265.

46. Steuer ER, Wordeman L, Schroer TA, Sheetz MP: **Localization of cytoplasmic dynein to mitotic spindles and kinetochores.** *Nature* 1990, **345**:266-268.

47. Starr DA, Williams BC, Hays TS, Goldberg ML: **ZW10 helps recruit**
•• **dynactin and dynein to the kinetochore.** *J Cell Biol* 1998, **142**:763-774.
In this important study, the authors establish a link between the kinetochore and cytoplasmic dynein through the interaction between the essential kinetochore component, ZW10, and the p50 subunit of dynactin. The authors conclude that while dynein may be involved in the coordination of chromosome separation, it is not involved in either the capture of spindle microtubules nor the congression of the metaphase chromosomes in *Drosophila*.

48. Saunders WS, Koshland D, Eshel D, Gibbons IR, Hoyt MA: ***Saccharomyces cerevisiae* kinesin- and dynein-related proteins required for anaphase chromosome segregation.** *J Cell Biol* 1995, **128**:617-624.

49. Kahana JA, Schlenstedt G, Evanchuk DM, Geiser JR, Hoyt MA, Silver PA: **The yeast dynactin complex is involved in partitioning the mitotic spindle between mother and daughter cells during anaphase B.** *Mol Biol Cell* 1998, **9**:1741-1756.

50. Campbell KS, Cooper S, Dessing M, Yates S, Buder A: **Interaction of**
• **p59(fyn) kinase with the dynein light chain, Tctex-1, and colocalization during cytokinesis.** *J Immunol* 1998, **161**:1728-1737.
Using the yeast two-hybrid system an interaction between a tyrosine kinase, p59^{fyn}, and the cytoplasmic dynein light chain (Tctex-1) was discovered. The striking co-localization of p59fyn kinase and cytoplasmic dynein at the cleavage furrow and the mitotic spindle during cytokinesis may suggest a recruiting or a regulatory mechanism for cytoplasmic dynein at these structures.

51. Tai AW, Chuang J-Z, Sung C-H: **Localization of Tctex-1, a cytoplasmic dynein light chain, to the Golgi apparatus and evidence for dynein complex heterogeneity.** *J Biol Chem* 1998, **273**:19639-19649.

52. Rappaport R: *Cytokinesis in Animal Cells.* Cambridge, UK: Cambridge University Press; 1996.

53. Wheatley SP, Wang Y-l: **Midzone microtubule bundles are continuously required for cytokinesis in cultured epithelial cells.** *J Cell Biol* 1996, **135**:981-989.

54. Giansanti MG, Bonaccorsi S, Williams B, Williams EV, Santolamazza C, Goldberg ML, Gatti M: **Cooperative interactions between the central spindle and the contractile ring during *Drosophila* cytokinesis.** *Genes Dev* 1998, **12**:396-410.

55. Schafer DA, Gill SR, Cooper JA, Heuser JE, Schroer TA: **Ultrastructural analysis of the dynactin complex: an actin-related protein is a component of a filament that resembles F-actin.** *J Cell Biol* 1994, **126**:403-412.

56. Holleran EA, Tokito MK, Karki S, Holzbaur ELF: **Centractin (ARP1) associates with spectrin revealing potential mechanism to link dynactin to intracellular organelles.** *J Cell Biol* 1996, **135**:1815-1829.

57. Williams BC, Riedy MF, Williams EV, Gatti M, Goldberg ML: **The *Drosophila* kinesin-like protein KLP3A is a midbody component required for central spindle assembly and initiation of cytokinesis.** *J Cell Biol* 1995, **129**:709-723.

58. Raich WB, Moran AN, Rothman JH, Hardin J: **Cytokinesis and midzone microtubule organization in *Caenorhabditis elegans* require the kinesin-like protein ZEN-4.** *Mol Biol Cell* 1998, **9**:2037-2049.

59. Adams RR, Tavares AVM, Salzberg A, Bellen HJ, Glover DM: ***pavarotti* encodes a kinesin-like protein required to organize the central spindle and contractile ring for cytokinesis.** *Genes Dev* 1998, **12**:1483-1494.

60. Harada AY, Takei Y, Kania Y, Tanaka Y, Nonaka S, Hirokawa N: **Golgi**
•• **vesiculation and lysosome dispersion in cells lacking cytoplasmic dynein.** *J Cell Biol* 1998, **141**:51-59.
This is the only study to date which analyzes a cytoplasmic dynein heavy chain (cDHC1) gene knockout in the mouse. The authors observed that transgenic mice lacking the dynein gene died early in embryogenesis. More detailed analysis of cDHC1$^{-/-}$ blastocyst cells cultured *in vitro* revealed that the Golgi, endosomes, and lysosomes in these cells had lost their characteristic perinuclear distribution and were instead distributed throughout the cytoplasm.

61. Presley JF, Cole NB, Schroer TA, Hirschberg K, Zaal KJ, Lippincott-
•• Schwartz J: **ER-to-Golgi transport visualized in living cells.** *Nature* 1997, **389**:81-85.
Using green fluorescent protein labeled vesicular stomatis viral glycoprotein (VSVG) as a cargo, the authors were able to track the movements of pre-Golgi structures from the endoplasmic reticulum (ER) to the Golgi. They observed that this ER-to-Golgi transport was significantly blocked by the overexpression of the p50 (dynamitin) subunit of dynactin, indicating a critical role for dynein and dynactin in the transport of these organelles.

62. Burkhardt JK, Echeverri CJ, Nilsson T, Vallee RB: **Overexpression of**
• **the dynamitin (p50) subunit of the dynactin complex disrupts dynein-dependent maintenance of membrane organelle distribution.** *J Cell Biol* 1997, **139**:469-484.
The authors followed up on the initial observation by Echeverri *et al.* [37] that p50 overexpression disrupts the dynein–dynactin interaction. In this study, they further analyze the effects of p50 overexpression on Golgi integrity and find that disruption of the dynein–dynactin interaction leads to the dispersion of the Golgi throughout the cytoplasm.

63. Lippincott-Schwartz J: **Cytoskeletal proteins and Golgi dynamics.** *Curr Opin Cell Biol* 1998, **10**:52-59.

64. Bingham JB, Schroer, TA: **Self-regulated polymerization of the actin-related protein Arp1.** *Curr Biol* 1999, in press.
•• The authors examine the *in vitro* polymerization dynamics of purified Arp1. They note that purified Arp1 assembles rapidly into short filaments, and that the dynamics of Arp1 polymerization differ significantly from those noted for actin.

65. 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.

66. Hirokawa N, Sato-Yoshitake R, Yoshida T, Kawashima T: **Brain dynein (MAP 1C) localizes on both anterogradely and retrogradely transported membranous organelles *in vivo*.** *J Cell Biol* 1990, **111**:1027-1037.

67. Waterman-Storer CM, Karki S, Kuznetsov SA, Tabb JS, Weiss DG,
•• Langford GM, Holzbaur EL: **The interaction between cytoplasmic dynein and dynactin is required for fast axonal transport.** *Proc Natl Acad Sci USA* 1997, **94**:12180-12185.
Using a polyclonal antibody to the p150^{Glued} subunit of dynactin that blocks the dynein–dynactin interaction on an affinity column, the authors investigate the effects of this antibody on microtubule-based motility in the extruded axoplasm from the squid giant axon. They observed that vesicle motility was inhibited in both directions, with the organelles and vesicles remaining firmly attached to the microtubules. The authors also noted that incubation with this antibody resulted in the loss of dynein from membranes, suggesting that dynactin mediates the association of the dynein with vesicles.

68. Ahmad FJ, Echeverri CJ, Vallee RB, Baas PW: **Cytoplasmic dynein
•• and dynactin are required for the transport of microtubule into the axon.** *J Cell Biol* 1998, **140**:391-401.
Due to the disruptive effect of p50 overexpression on the dynein–dynactin interaction, p50 (dynamitin) has become an important tool in probing the cellular function of cytoplasmic dynein. In this study, the authors examine the effects of p50 overexpression on the translocation of microtubules down the axon. They report that in the presence of excess p50 microtubules failed to transport to the neurite and remained near the centrosome.

69. Swaroop M, Garen A: **Sequence analysis of the complete cDNA and encoded polypeptide for the *Glued* gene of *Drosophila melanogaster*.** *Proc Natl Acad Sci USA* 1987, **84**:6501-6505.

70. Holzbaur ELF, Hammerback JA, Paschal BM, Kravit NG, Pfister KK, Vallee RB: **Homology of a 150K cytoplasmic dynein-associated polypeptide with the *Drosophila* gene *Glued*.** *Nature* 1991, **351**:579-583.

71. Plough HH, Ives PT: **Induction of mutations by high temperature in *Drosophila*.** *Genetics* 1935, **20**:42-69.

72. Gepner J, Li M, Ludmann S, Kortas C, Boylan K, Iyadurai SJ, McGrail M, Hays TS: **Cytoplasmic dynein function is essential in *Drosophila melanogaster*.** *Genetics* 1996, **142**:865-878.

73. Fan S-S, Ready DF: ***Glued* participates in distinct microtubule-
• based activities in *Drosophila* eye development.** *Development* 1997, **124**:1497-1507.
In this systematic study of the development of the *Drosophila* compound eye in the presence of dominant negative Glued^{1} mutation, the authors describe how Glued is involved in discrete stages of the eye development.

74. Reddy S, Jin P, Trimarchi J, Caruccio P, Phillis R, Murphey RK: **Mutant
• molecular motors disrupt neural circuits in *Drosophila*.** *J Neurobiol* 1997, **33**:711-723.
The authors examined the neuronal structure in *Drosophila* with the dominant-negative mutation in Glued (*Gl ^{1}*) and found that the axonal anatomy was disrupted. They further observed that the disruption in neuronal structure corresponded to disruption in a reflex response, suggestive of a disruption in synaptic connectivity.

75. Phillis R, Statton D, Caruccino P, Murphey RK: **Mutations in the 8 kDa dynein light chain gene disrupt sensory axon projections in the *Drosophila* imaginal CNS.** *Development* 1996, **122**:2955-2963.

76. Huntington's Disease Collaborative Research Group: **A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes.** *Cell* 1993, **72**:971-983.

77. Li X-J, Li S-H, Sharp AH, Tokito M, Lanahan A, Worley P, Holzbaur ELF, Ross CA: **A huntingtin-associated protein enriched in brain with implications for pathology.** *Nature* 1995, **378**:398-402.

78. Engelender S, Sharp AH, Colomer V, Tokito MK, Lanahan A, Worley P, Holzbaur EL, Ross CA: **Huntingtin-associated protein 1 (HAP1) interacts with the p150^{Glued} subunit of dynactin.** *Hum Mol Genet* 1997, **6**:2205-2212.

79. Li S-H, Gutekunst C-A, Hersch SM, Li X-J: **Interaction of huntigtin-associated protein with dynactin p150^{Glued}.** *J Neurosci* 1998, **18**:1216-1269.

80. Tokito MK, Holzbaur ELF: **The genomic structure of DCTN1, a candidate gene for limb-girdle muscular dystrophy (LGMD2B).** *Biochem Biophys Acta* 1998, **1442**:432-436.

81. Kopec K, Chambers JP: **Effect of Alzheimer's brain extracts on dynein immunoreactivity in PC12 cells.** *Proc Soc Exp Biol Med* 1997, **216**:429-437.

82. Sodeik B, Ebersold MW, Helenius A: **Microtubule-mediated
• transport of incoming herpes simplex virus 1 capsids to the nucleus.** *J Cell Biol* 1997, **136**:1007-1021.
This paper describes the dependence of the transport of herpes simplex virus (HSV)1 capsids to the nucleus on the microtubule-based system. Since dynein was found to be associated with viral capsids following infection of fibroblasts, the authors suggest that this virus may use the retrograde organelle transport system to efficiently infect its host.

83. Gibbons IR, Gibbons BH, Mocz G, Asai DJ: **Multiple nucleotide-binding sites in the sequence of dynein β heavy chain.** *Nature* 1991, **352**:640-643.

84. Ogawa K: **Four ATP-binding sites in the midregion of the beta heavy chain of dynein.** *Nature* 1991, **352**:643-645.

85. Gee MA, Heuser JE, Vallee RB: **An extended microtubule-binding
•• structure within the dynein motor domain.** *Nature* 1997, **390**:636-639.
This paper describes an unusual microtubule-binding domain in cytoplasmic dynein observed by ultrastructural analysis using electron microscopy. The authors predict that the microtubule-binding domain forms a hairpin-like stalk which is well separated from the ATPase domain, suggesting that dynein may use a force generation mechanism which is distinct from that of myosin and kinesin.

86. Samso M, Radermacher M, Frank J, Koonce MP: **Structural characterization of a dynein motor domain.** *J Mol Biol* 1998, **276**:927-937.

87. Paschal BM, Holzbaur EL, Pfister KK, Clark S, Meyer DI, Vallee RB: **Characterization of a 50 kDa polypeptide in a cytoplasmic dynein preparations reveals a complex with p150^{Glued} and a novel actin.** *J Biol Chem* 1993, **268**:15318-15323.

88. Reilein AR, Tint IS, Peunova NI, Enikolopov GN, Gelfand VI: **Regulation of organelle movement in melanophores by protein kinase A (PKA), protein kinase C (PKC), and protein phosphatase 2A (PP2A).** *J Cell Biol* 1998, **142**:803-813.

89. Farshori P, Holzbaur ELF: **Dynactin phosphorylation is modulated in response to cellular effectors.** *Biochem Biophys Res Comm* 1997, **232**:810-816.

90. Niclas J, Allan VJ, Vale RD: **Cell cycle regulation of dynein association with membranes modulates microtubule-based organelle transport.** *J Cell Biol* 1996, **133**:585-593.

91. Schwartz K, Richards K, Botstein D: **BIM1 encodes a microtubule-binding protein in yeast.** *Mol Biol Cell* 1997, **8**:2677-2691

92. Berrueta L, Kraeft SK, Tirnauer JS, Schuyler SC, Chen LB, Hill DE, Pellman Bierer BE: **The adenomatous polyposis coli-binding protein EB1 is associated with cytoplasmic and spindle microtubules.** *Proc Natl Acad Sci USA* 1998, **95**:10596-10601.

93. Yeh E, Skibbens RV, Cheng JW, Salmon ED, Bloom K: **Spindle dynamics and cell cycle regulation of dynein in budding yeast, *Saccharomyces cerevisiae*.** *J Cell Biol* 1995, **130**:687-700.