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Microtubule-based transport in filamentous fungi

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Defects in microtubule-based transport are implicated in many neuropathologies. The filamentous fungi *Aspergillus nidulans* and *Ustilago maydis* are valuable models for studying transport owing to their yeast-like genetic and biochemical tractability and metazoan-like dependence on microtubule-based transport for cellular trafficking. In these organisms the role of microtubules in nuclear positioning is well studied, but recent work has expanded the range of cargos to include endosomes, messenger RNA, secretory vesicles, peroxisomes, and nuclear pore complexes, reflecting the diversity of metazoan systems. Furthermore, similarities in transport mechanisms exist between filamentous fungi and metazoan neurons, demonstrating the suitability of *A. nidulans* and *U. maydis* for studying the molecular basis of transport-related neuropathologies such as lissencephaly, motor neuron disease, and Perry syndrome.

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Introduction

The size and polarization of many eukaryotic cells demands a system of fast, directed, and long-range transport of cellular cargos that typically occurs along microtubules. Defects in microtubule-based transport are pervasive in neurodegenerative and neurodevelopmental disorders [1], necessitating the development of tractable model systems to study transport processes. Several species of filamentous fungi have emerged as powerful model organisms for such purposes, owing to their long hyphal compartments that grow via microtubule-dependent transport of cellular cargos that include endosomes, peroxisomes, nuclei, and mRNA. In this review we focus on the fungal species *Ustilago maydis* and *Aspergillus nidulans*. *U. maydis* is a plant pathogen that exhibits both yeast-like cell division by budding and

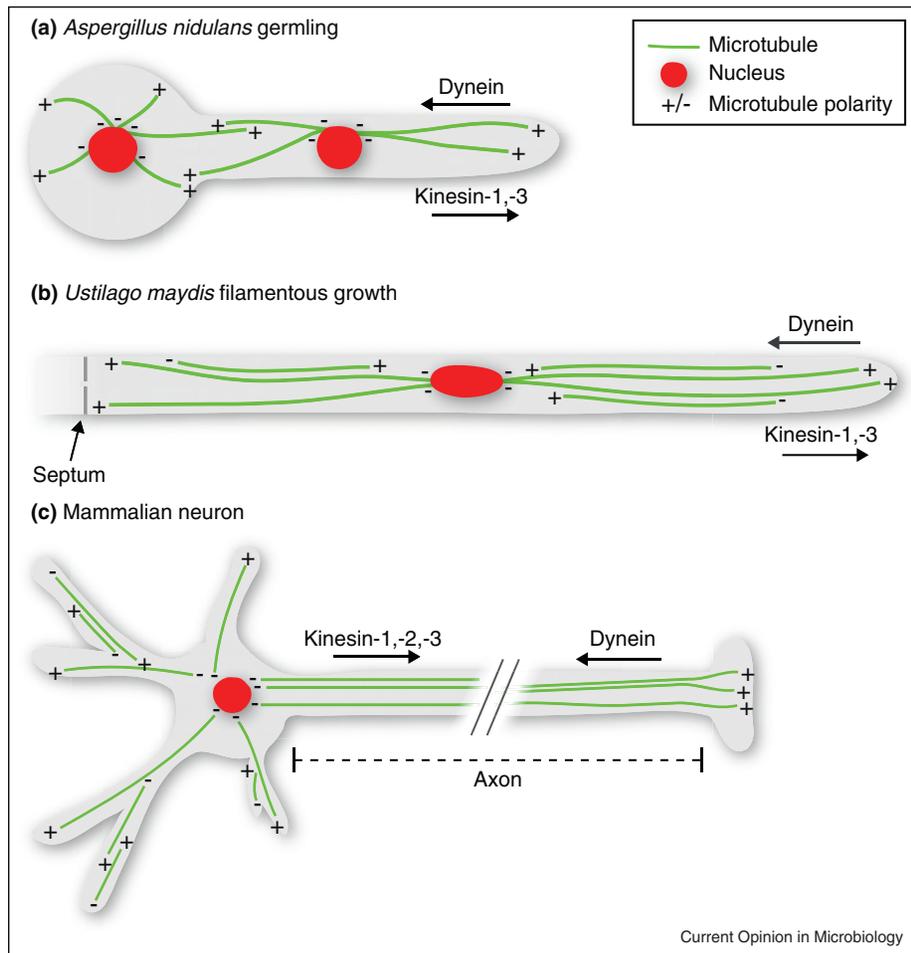
dikaryotic hyphal growth. In contrast, *A. nidulans* grows only as long polarized hyphae that can be maintained in either haploid or diploid states. Importantly, both systems have genetic tractability on par with that of *Saccharomyces cerevisiae* [2,3] and fully sequenced and annotated genomes [4,5]. Exploitation of these traits in recent years has yielded significant advances in our understanding of transport regulation, demonstrating the broad utility of filamentous fungi in understanding microtubule-based transport.

Microtubule-based motors and their cargos

Like the dendrites and axons of metazoan neurons, both *U. maydis* and *A. nidulans* have discrete regions in which polarized microtubules are oriented in either anti-parallel or uniform arrays (Figure 1). Kinesin motors carry out plus-end-directed (anterograde) transport, while minus-end-directed (retrograde) transport is mediated by dynein. Humans encode 45 kinesins, of which 15 are members of the kinesin-1, kinesin-2, or kinesin-3 families used for transport of organelles and other cargo [6,7]. The genomes of filamentous fungi typically contain between 10 and 12 kinesins with 2–3 members of the kinesin-1 and kinesin-3 families participating in transport [8]. While multiple kinesin motors are used for anterograde motility, nearly all retrograde cytoplasmic movement in both metazoans and filamentous fungi is driven by a single cytoplasmic dynein motor (referred to here as dynein). Dynein activity is regulated by an array of associated proteins and protein complexes, including dynactin, Lis1, Nudel, and dynein-associated subunits, perhaps in order to achieve functional diversity [9]. Although *S. cerevisiae* encodes the basic dynein machinery (used exclusively for nuclear migration), it uses actin-based transport for most cellular trafficking [10] and lacks cargo-transporting kinesins along with several components of the dynactin complex and some dynein light chains that are shared between filamentous fungi and metazoans (Figure 2). Thus, filamentous fungi are ideal model systems for investigating the conserved microtubule-based transport processes of complex multicellular eukaryotes.

Nuclei were the first microtubule-dependent cargo described in filamentous fungi [11] and studies in these organisms continue to provide important insight into the mechanism of nuclear distribution. The list of cellular cargos transported by microtubule-based motors in filamentous fungi has since grown considerably, and it is probable that additional fungal cargos remain to be discovered. Here, we highlight the expanding diversity of known cargos for dynein and kinesin in *A. nidulans* and *U. maydis*.

Figure 1



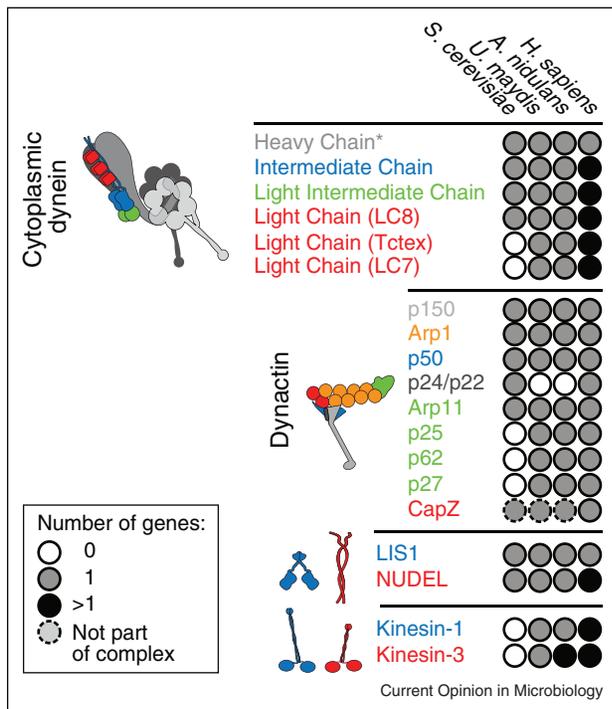
Microtubule organization and motor composition in filamentous fungi hyphae and mammalian neurons. **(a)** *Aspergillus nidulans* grows by rapid tip extension forming polarized multinucleate hyphae. The region from the last nucleus to the hyphal tip contains uniformly orientated microtubule arrays, with their plus ends pointing toward the direction of growth and their minus ends anchored at the spindle pole body/microtubule organizing center. Kinesin-1 and kinesin-3 motors drive plus-end-directed movement, while dynein drives movement toward the microtubule minus end. Regions between nuclei contain microtubules of mixed polarity. **(b)** The dimorphic plant pathogenic fungus *Ustilago maydis* undergoes conditional filamentous growth resulting in highly polarized uniuucleate infectious hyphae. Microtubules in *U. maydis* span the length of the infectious hyphae, but only regions $\sim 12 \mu\text{m}$ from either the septum or the hyphal tip contain uniformly oriented microtubules. As in *A. nidulans*, kinesin-1 and kinesin-3 support plus-end-directed cargo transport, while dynein moves cargo toward the minus ends of microtubules. **(c)** Mammalian neurons contain unipolar microtubules within axons, with their plus ends oriented toward the synaptic terminal. Transport toward the synapse is mediated by kinesin-1, kinesin-2, and kinesin-3, while transport toward the cell body is largely dynein-driven. Microtubules are anti-parallel in dendrites.

Endosomes

It is well established that dynein and kinesin-3 support the motility of early endosomes in filamentous fungi, which is thought to facilitate long-range communication between the nucleus and the growing hyphal tip [12,13^{••},14,15^{••},16]. This makes endosome trafficking a valuable tool in dissecting bi-directional transport and its regulation. In *U. maydis* hyphae, which contain both unipolar and anti-parallel microtubule arrays (Figure 1), recent work shows that dynein moves endosomes through unipolar regions, while kinesin-3 is used in anti-parallel regions for further transport toward the nucleus [17^{••}]. Thus, cooperation between opposite-polarity motors

supports the transport of endosomes across the entire length of the cell. In *A. nidulans*, which also uses dynein and kinesin-3 for endosome transport [13^{••},14,15^{••},16], the p25 subunit of the dynactin complex is required for the physical interaction of dynein with early endosomes [15^{••}], demonstrating a cargo adaptor role for dynactin in filamentous fungi that is consistent with reported roles for dynactin as a cargo adaptor in metazoans [18,19]. Post-translational tubulin modifications may provide another layer of regulation for endosome traffic. In *A. nidulans*, endosome-transporting kinesin-3/UncA was found to prefer a subpopulation of detyrosinated microtubules [20,21]. Intriguingly, a similar

Figure 2



Genes and subunit composition of microtubule motors and their accessory factors in *S. cerevisiae*, *U. maydis*, *A. nidulans*, and humans. The number of genes found in each organism is color-coded as shown in the legend. *S. cerevisiae* lacks orthologues of 2 of the 3 dynein light chains, and 3 of the 4 dynactin subunits that form the pointed end complex of the Arp1 filament (shown in green), probably reflecting the fact that dynein does not participate in vesicular transport in this system. *S. cerevisiae* also lacks kinesin-1 and kinesin-3 motors, instead relying on actin-based myosins for intracellular transport. By contrast, *U. maydis* and *A. nidulans* contain orthologues of most or all human dynein and dynactin subunits with the exception of p24/p22, and use kinesin-1 and kinesin-3s for cargo transport, making them excellent systems for dissecting the regulation of microtubule-based transport. *The single complete dynein heavy chain in *U. maydis* is encoded by two genes [59].

preference for detyrosinated microtubules allows kinesin-1, but not kinesin-3, to discriminate between axons and dendrites in mammalian neurons [22,23].

Messenger RNA

Microtubule motor-driven transport and subsequent localized translation of mRNA is an important feature of eukaryotic biology. Recently, microtubule-based mRNA transport was also reported in *U. maydis*, where the RNA binding protein Rrm4 mediates the recognition and shuttling of target mRNAs [24]. In this study, Rrm4 recognized target mRNAs by a 'zipcode' sequence in the 3'-UTR of the transcript, promoting the packaging of the mRNA into ribonucleoprotein complexes (mRNPs) for subsequent delivery [24]. This is reminiscent of the mechanism by which β -actin mRNA is transported to the cell periphery via microtubules in mammalian systems, reflecting the probable conservation of

microtubule-based mRNA transport mechanisms between filamentous fungi and metazoans [25]. A subsequent study showed that bi-directional Rrm4-mediated mRNA transport is driven by dynein and kinesin-3 [26**]. Interestingly, Rrm4-containing mRNPs also required functional endosomes for microtubule-based movement, suggesting that these mRNPs may hitch a ride on dynein and kinesin-3-driven early endosomes [26**]. A similar relationship between membrane trafficking and localized mRNA has been proposed in *S. cerevisiae* [27]. The significance of this mRNA transport in *U. maydis* was highlighted in another recent study demonstrating that efficient secretion of an endochitinase, Cts1, is dependent on Rrm4-mediated mRNA shuttling [28].

Secretory vesicles

In mammalian systems, a large and diverse array of cellular cargos have been identified for kinesin-1 [7], while in filamentous fungi organellar kinesin-1 cargos have remained elusive. However, a recent study in *U. maydis* supports a direct role for kinesin-1 in polarized secretion [29**]. Kinesin-1, along with the actin-based motor myosin-5, is needed for the delivery of chitin-synthase-containing vesicles (CSVs) to the polarized growth region for exocytic secretion [29**]. In this pathway, retrograde dynein activity probably functions in the removal of excess CSVs from the region of polarized growth, therein generating a gradient of chitin synthase for secretion [29**].

Peroxisomes

Peroxisomes are ubiquitous single membrane-bound organelles that perform important metabolic functions. In mammalian cells, a small population of peroxisomes undergo long-range dynein and dynactin dependent saltatory movements [30]. In *A. nidulans*, peroxisome behavior is strikingly similar, with small populations undergoing dynein and kinesin-3 dependent bi-directional movements, while the majority remain immotile [13**]. Recent work in *A. nidulans* suggests that a subpopulation of peroxisomes may act as vehicles for transporting proteins associated with microtubule-organizing centers (MTOCs) [31*], therefore the idea of some cargos 'piggy-backing' on others for transport may not be limited to the relationship between mRNPs and endosomes. In yeast, actin-based transport of peroxisomes to the bud neck during cell division is thought to facilitate equal inheritance of the organelle between mother and daughter cells [10]. In filamentous fungi, peroxisome transport probably serves a similar function, as the distribution of the organelle must keep pace with the growing hyphal tip.

Nuclear pore complexes

Nuclear pore complexes (NPCs) were also recently identified as microtubule-based cargos in *U. maydis*, which, along with other fungi, lacks nuclear lamina [32*]. The NPCs displayed dynein and kinesin-1 dependent movements within the nuclear envelope that were

required for both NPC and chromatin distribution [32[•]]. These results raise the interesting possibility that microtubule-dependent NPC positioning may play a role in transcriptional regulation.

Transport initiation at microtubule plus ends

In filamentous fungi, as well as in metazoans, dynamic microtubule plus ends are sites for cargo loading and transport initiation [12,13^{••},33,34]. Consistent with this idea, dynein and/or its regulators accumulate at microtubule plus ends in many cell types [9]. Here we highlight recent progress made in filamentous fungi toward understanding how dynein gets to and is retained at microtubule plus ends, and how it is subsequently loaded onto cargo.

In both *U. maydis* and *A. nidulans*, dynein is recruited to the dynamic plus ends of cytoplasmic microtubules in a kinesin-1-dependent manner [12,13^{••},35]. In *U. maydis* an interaction between the end-binding protein, EB1, and dynactin is important for retention of a subpopulation of dynein [36^{••}], though other mechanisms are likely to be involved (see below). Ultimately, this plus end recruitment generates spatially confined dynein pools that are proposed to function as ‘loading zones’ for the capture of incoming kinesin-3-driven early endosomes [12,36^{••}]. Evidence suggests similar spatial loading of dynein onto endosomes at microtubule plus ends in *A. nidulans* [13^{••}], though the situation in *U. maydis* may be more complex, as dynein can also be loaded onto moving endosomes before they reach the established dynein ‘loading zone’ at the hyphal tip [37^{••}].

All of the known cellular functions of dynein, including its accumulation at the microtubule plus end, are dependent on the largest subunit of the dynactin complex, p150^{Glued} (referred to here as p150). The N-terminus of p150 contains a conserved microtubule-binding region consisting of a cytoskeleton-associated protein glycine rich (CAP-Gly) domain, immediately followed by a region rich in basic amino acids. CAP-Gly domains have been shown to interact directly with EEY/F-COO⁻ motifs, which are sequence elements found in α -tubulin and microtubule end-binding proteins, such as EB1 [38]. A recent functional analysis of *A. nidulans* p150 demonstrated that the CAP-Gly domain is not required for the microtubule plus end localization of p150 [39^{••}]. Instead, the basic domain was necessary for the plus end localization of both p150 and dynein, and essential for early endosome motility [39^{••}]. Furthermore, the basic domain was required for the interaction of p150 with microtubules *in vivo*, which may be important for the kinesin-1-dependent recruitment of dynein and dynactin to microtubule plus ends in *A. nidulans* [39^{••}].

An additional layer of regulation may be to inhibit dynein activity at microtubule plus ends before cargo loading. For example, in both *A. nidulans* and *U. maydis* the

orthologue of Lis1 appears to play an important role in initiating dynein-mediated transport from the microtubule plus end and may function in loading dynein onto cargos to initiate retrograde motility [12,13^{••}]. In *A. nidulans*, in the absence of Lis1 both dynein and its cargos leave plus ends with a dramatically reduced frequency, but moving molecules or cargos do so at normal speeds [13^{••}] (Figure 3). Further support for a transport initiation role for Lis1 comes from experiments in *S. cerevisiae*, where dynein positions daughter nuclei by pulling on nucleus-attached astral microtubules while anchored to the cell cortex [40]. Here, Lis1/Pac1 is required for dynein plus end localization and subsequent ‘off-loading’ to the cell cortex [40], a scenario that is analogous to loading dynein onto organellar cargo. Recent work suggests a molecular mechanism for how Lis1 regulates dynein [41^{••}]. In this work, single-molecule motility assays and single-particle electron microscopy suggested that Lis1 binds at the junction of the dynein ATPase ring and microtubule-binding stalk. When bound to dynein, Lis1 appeared to sever communication between these domains, causing dynein to become anchored to the microtubule [41^{••}]. Thus, by binding dynein, Lis1 may increase the residence time of dynein at the microtubule plus end, and assist in the kinetics of cargo loading [41^{••}].

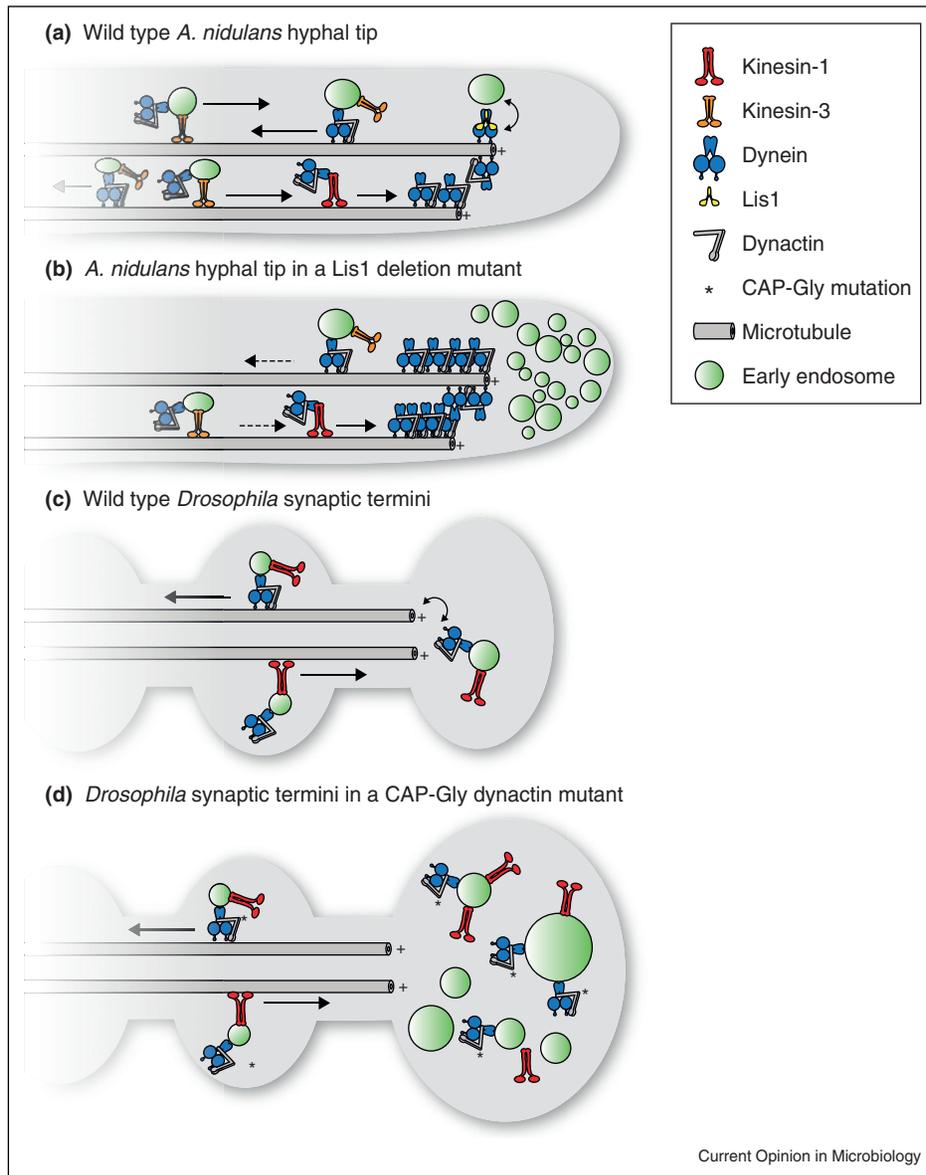
Molecular motor recycling

Many eukaryotic cell types exhibit a mutual interdependence between opposite polarity motors for bi-directional motility (discussed recently in [13^{••}]). How molecular motors coordinate with each other to achieve this interdependence is an area of active inquiry, but recent work suggests a mechanism of motor ‘recycling’ in which dynein and kinesin themselves function as cargo for an opposite polarity motor, facilitating their return to their respective starting points on the microtubule track. In both *A. nidulans* and *U. maydis*, as well as in mammalian neurons, the plus end localization of dynactin, and thus proper retrograde transport, is dependent on kinesin-1 [12,13^{••},35,42[•]]. Similarly, a *Drosophila* kinesin-1 orthologue functions synergistically with plus end localized dynactin at presynaptic axon terminals to initiate retrograde transport [43[•]]. Demonstrating the reciprocity of this relationship, the genetic absence of dynein in *A. nidulans* causes kinesin-3 accumulation at microtubule plus ends, while in *Drosophila* neurons, kinesin-1 accumulates at axon terminals as a consequence of dynactin dysfunction [13^{••},43[•]]. Collectively, these experiments provide compelling evidence for a kind of molecular conveyor belt on which dyneins and kinesins are continuously recycled between opposite microtubule ends.

Filamentous fungi as models for understanding disease

Defects in microtubule-based transport are associated with several neuropathologies [1]. One such pathology, lissencephaly, is caused by dominantly inherited loss of

Figure 3



Models for transport initiation in *A. nidulans* and *Drosophila*. **(a)** In wild type *A. nidulans* hyphae, dynein accumulates at dynamic microtubule plus ends in a process dependent on kinesin-1. Efficient loading of dynein onto early endosomes requires the accessory protein Lis1. **(b)** In the absence of Lis1, dynein and cargos accumulate aberrantly at the hyphal tip [12,13**,35]. Reflecting a role for Lis1 in loading dynein onto endosomes, the frequency but not velocity of endosome movements is dramatically affected by the deletion of Lis1. **(c)** In *Drosophila* neurons the CAP-Gly domain of the p150 subunit of the dynactin complex is required for the initiation of dynein-dependent vesicle transport from axonal termini. **(d)** Introduction of a loss-of-function and motor neuron disease-associated mutation within the CAP-Gly domain causes accumulation of cargo, yet axonal transport is unperturbed [43*].

function mutations in the human *LIS1* gene [44]. The connection between Lis1 and dynein was first discovered in *A. nidulans*, in which loss-of-function mutants of the *LIS1* orthologue (*nudF*) phenocopy the severe nuclear distribution and growth defects of dynein loss-of-function mutants [45]. The identification of a point mutation (R3086C) in AAA4 of the dynein motor domain that

suppressed the Lis1 null phenotype in *A. nidulans* provided a clue regarding the relationship between these proteins [46,47]. In addition to mitigating the nuclear migration defects of the Lis1 null strain, the suppressor mutation induced a dynein localization pattern of discrete, nearly immobile puncta along microtubules [46]. When mapped onto recent high-resolution dynein crystal

structures [48,49], the mutated residue appeared to form an 'arginine finger' motif, which probably transmits conformational changes within the dynein ATPase motor ring [41**]. Interestingly, analogous mutations in yeast dynein mimicked regulation by Lis1 (see above), corroborating its proposed role in regulating allosteric communication within the dynein motor [41**].

In addition to lissencephaly, filamentous fungi are poised to broaden our understanding of microtubule-based transport in motor neuron diseases and the Parkinson's-like Perry syndrome, in which mutations in both dynein and dynactin have been identified in human patients [1,50]. In particular, the functional integrity of the CAP-Gly domain of the dynactin subunit p150 appears to be an important mediator of disease. Causative mutations for these divergent disorders reside less than 15 residues apart in p150's CAP-Gly domain, indicating that CAP-Gly dysfunction may promote pathology by multiple mechanisms. Despite this, the CAP-Gly domain appears to be dispensable for microtubule-based transport processes in several experimental systems. As described above, in *A. nidulans* deletion of the CAP-Gly domain yields normal growth and nuclear migration phenotypes and does not perturb the retrograde velocities of motile endosomes, or the frequency with which they leave the hyphal tip [39**]. Similarly, depletion of the CAP-Gly domain in non-neuronal mammalian and *Drosophila* cells is not deleterious for organelle transport or distribution [51,52]. In *S. cerevisiae* the CAP-Gly domain was found to be important for initiating dynein-mediated movement of dividing nuclei into the bud neck, but dispensable for dynactin-mediated increases in dynein processivity *in vitro* [53,54]. These studies suggest that the functional role of the CAP-Gly domain may be subtle or exclusive to high load functions, such as nuclear positioning. Consistent with its proposed role in yeast, recent studies in *Drosophila* and mouse neurons suggest that the p150 CAP-Gly domain is important for retrograde transport initiation, but not transport itself [42*,43*]. In these systems the CAP-Gly domain is dispensable for cargo flux, velocity, and run-length within the axon but required to enrich the dynactin complex and promote retrograde transport from synaptic terminals (Figure 3).

Thus, both dynactin and Lis1 are important for transport initiation (Figure 3). While the connection between dynactin and Lis1 in transport initiation is currently unclear, their mechanisms are probably different, making this an exciting area for future study. One possibility is that Lis1 and dynactin may have cell-type or organism-specific functions. In filamentous fungi and in non-neuronal mammalian cells, both dynactin and dynein localize to microtubule plus ends [9,12,13**,35]. However, in mammalian neurons only dynactin plus end localization is observed, perhaps because dynein

recruitment to axon terminals is rate limiting for transport in neurons, whereas other factors such as the availability of cargo, may be rate limiting in other cell types [42*]. In neurons, p150 may serve to concentrate dynactin, which is essential for dynein function *in vivo*, for efficient and immediate engagement in retrograde motility once motors become available.

Another possibility is that dynactin and Lis1 are not mutually exclusive in transport initiation, but rather cooperative. In both *S. cerevisiae* and *A. nidulans*, Lis1 is proposed to 'prime' dynein for cargo loading at microtubule plus ends [13**,41**]. Once loaded onto the cell cortex in yeast (which effectively functions as dynein cargo), the activity of the p150 CAP-Gly domain is important in initiating, but not sustaining, the dynein-driven microtubule sliding that positions the daughter nucleus [54], perhaps through the displacement of Lis1 from the dynein complex [55,56]. This is consistent with the observed absence of Lis1 on retrograde cargo leaving the microtubule plus ends in *A. nidulans* [13**]. The loading and transport of diverse cellular cargos by only a single retrograde motor are likely to be highly regulated processes mediated by the sequential or combinatorial activities of many factors, including Lis1 and dynactin.

Conclusion

Many questions remain regarding the regulation of microtubule-based transport and how defects in this regulation lead to disease in humans. From the expansion of known cargos to the elucidation of retrograde transport initiation mechanisms, recent work in both *U. maydis* and *A. nidulans* demonstrates the utility of these organisms in understanding evolutionarily conserved microtubule-based transport processes. Moving forward, the biochemical and genetic tractability of filamentous fungi give them tremendous potential in understanding motor mechanisms as well as for high-throughput screening to identify novel drug targets and therapeutics. Recent descriptions of tetracycline-inducible expression systems for a related *Aspergillus* species, *Aspergillus niger*, as well as protocols for the purification of enzymatically active proteins from *A. nidulans* emphasize the suitability of filamentous fungi for heterologous protein expression and *in vitro* characterization [57,58]. Furthermore, the improving annotation of fungal genomes and feasibility of whole-genome sequencing makes these organisms amenable to the identification of novel genes involved in microtubule-based transport through genetic screens. With these strengths, future work, both *in vivo* and *in vitro*, is likely to lean heavily on conclusions drawn from filamentous fungi.

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- Evidence suggests that kinesin-1 in filamentous fungi is important in several secretory pathways. The authors demonstrate that kinesin-1 and myosin-5 mediate the transport of vesicles containing chitin synthase to the growing hyphal tip for exocytosis, while dynein returns these vesicles to the cell center if unsuccessfully secreted. This provides insight not only into the emerging role of kinesin-1, but also into how microtubule-based motors coordinate with actin-based transport mechanisms.
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Relatively little is known about the role of peroxisome transport in filamentous fungi. The authors of this study identify a subpopulation of peroxisomes to which components of microtubule-organizing centers (MTOCs) localize, leading to the hypothesis that transport of these peroxisomes facilitates the generation and maintenance of MTOCs at septa within *A. nidulans* hyphae.

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This paper describes dynein and kinesin-1 driven motility of nuclear pore complexes (NPCs) within the membranes of *U. maydis* nuclei. This motility was found to be important in maintaining a uniform distribution of NPCs in the nuclear membrane and for the proper trafficking of cellular contents between the nucleus and the cytoplasm. Additionally, the observation that NPC motility and distribution maintains chromosomal organization within the nucleus presents the exciting possibility of motor-based genetic regulation in filamentous fungi.

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The localization and subsequent activation of retrograde transport machinery at microtubule plus ends has been an active topic of pursuit in several model systems during the past year. This paper quantitatively describes the active retention of approximately half of the dynein motors by EB1 at the hyphal tip of filamentous *U. maydis*, with the remaining half being retained stochastically by a motor crowding mechanism at microtubule plus ends.

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How opposite polarity motors on the same cargo coordinate to achieve directed transport is not well understood. In this paper, the authors find that directionality of endosomes in *U. maydis* is dictated by the presence or absence of a single dynein motor on cargos to which multiple kinesin-3 motors are constitutively bound. Additionally, the observation that anterograde cargos change direction prior to reaching the dynein pool at the hyphal tip led the authors to hypothesize that dynein released from microtubule plus ends can load cargo 'on the run'.

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The *in vivo* role of the CAP-Gly domain of p150 is emerging, but still mysterious. In this paper, the authors find the CAP-Gly domain to be dispensable for endosome transport, distribution, and plus end tracking of dynein and dynactin in *A. nidulans*. Instead, the authors determined the basic domain that follows the CAP-Gly domain to be important for these functions, suggesting that the *in vivo* significance of the basic domain's microtubule binding capacity may be underemphasized.

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How Lis1 regulates dynein is of great interest as mutations in Lis1 cause lissencephaly in humans. This study combines structural, biochemical,

and single-molecule experiments with purified yeast dynein to demonstrate that Lis1 binding near AAA3 and AAA4 of the dynein motor domain disrupts allosteric communication between the microtubule-binding domain and ATPase domain, effectively anchoring the motor in a high microtubule affinity state despite continued hydrolysis of ATP.

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This paper supports a role for the CAP-Gly domain of p150 in retrograde transport initiation in mammalian neurons. Metrics for the integrity of axonal transport were largely normal in cells depleted of the CAP-Gly domain with the exception of a decrease in flux from the axon terminal. The CAP-Gly domain was found to enrich dynactin at microtubule plus ends, facilitating efficient transport from the terminal. Additionally, the authors propose mechanisms by which mutations in different regions of the CAP-Gly domain cause motor neuron disease or Perry syndrome.

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Using *Drosophila* neurons, this paper provides additional evidence for the regulation of transport initiation by the CAP-Gly domain of p150. With CAP-Gly utility impaired by a loss-of-function point mutation associated with motor neuron disease, the authors observe normal organelle trafficking within axons, but decreased retrograde flux from axon terminals. Coupled with the observed accumulation of cargo and dynein heavy chain at these axon terminals, this points to an inability of dynein to engage the microtubule track at plus ends in the absence of a functional CAP-Gly domain.

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