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Cytoplasmic dynein and early endosome transport

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Cytoplasmic dynein and early endosome transport

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Running title:

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Abstract

Microtubule-based distribution of organelles/vesicles is crucial for the function of many types of eukaryotic cells and the molecular motor cytoplasmic dynein is required for transporting a variety of cellular cargos toward the microtubule minus ends. Early endosomes represent a major cargo of dynein in filamentous fungi, and dynein regulators such as LIS1 and the dynactin complex are both required for early endosome movement. In fungal hyphae, kinesin-3 and dynein drive bi-directional movements of early endosomes. Dynein accumulates at microtubule plus ends; this accumulation depends on kinesin-1 and dynactin, and it is important for early endosome movements towards the microtubule minus ends. The physical interaction between dynein and early endosome requires the dynactin complex, and in particular, its p25 component. The FTS-Hook-FHIP (FHF) complex links dynein-dynactin to early endosomes, and within the FHF complex, Hook interacts with dynein-dynactin, and Hook-early endosome interaction depends on FHIP and FTS.

Key words:

Dynein, kinesin-1, kinesin-3, dynactin, LIS1, early endosome, FTS-Hook-FHIP, fungi

Introduction

Eukaryotic cells engulf material via endocytosis, a process of internalizing portions of the cell's plasma membrane and taking in nutrients [1-5]. Endocytic vesicles fuse with early endosomes, a class of organelles in which endocytic cargos are sorted into different cellular compartments to undergo eventually either degradation or recycling back to the plasma membrane [6, 7]. In filamentous fungi and metazoan cells, early endosomes undergo microtubule-dependent movements [6, 8]. These movements are particularly robust in elongated fungal hyphae, making filamentous fungi excellent systems for studying the underlying mechanisms [8-10]. In this review, we will discuss the mechanism of early endosome transport in filamentous fungi, which is microtubule-based and requires the molecular motors, cytoplasmic dynein and kinesin-3. The dynein regulators dynactin and LIS1 are also critical for the transport [9-12]. We will review the current mechanistic understanding of these proteins. We will discuss in detail the functions of these dynein regulators in fungal early endosome movement including their roles in the microtubule-plus-end accumulation of dynein, dynein-early endosome interaction and dynein-microtubule interaction. Finally, we will discuss how early endosomes are attached to dynein via an adaptor complex Hook-FTS-FHIP [13-15].

Cytoplasmic dynein and kinesin-3 drive bidirectional movements of early endosomes

In a typical eukaryotic cell during interphase, microtubules are polarized such that their minus-ends are embedded in microtubule-organizing centers (MTOC) near the nucleus and their dynamic plus ends extend toward the cell periphery [16-20]. Cytoplasmic dynein, a minus-end-directed microtubule-based motor, drives the movements of a variety of cargoes from cell periphery towards the cell center [6, 12, 21-23]. In the hyphae of fungal organisms such as *Aspergillus nidulans* and *Ustilago maydis*, microtubules in the hyphal tip region are polarized such that their plus ends extend toward the apical dome cortex and their minus ends are at the spindle-pole body, the main MTOC in fungi (Figure 1) [24-33]. Cytoplasmic dynein and the plus-

end-directed kinesin-3 drive bi-directional movements of early endosomes in fungi and metazoan [6, 34-36]. In *U. maydis* and *A. nidulans*, kinesin-3 drives early endosome movements toward the hyphal tip, whereas dynein drives them away from the hyphal tip, and therefore, an abnormal accumulation of early endosomes at the hyphal tip can be seen in mutants defective in dynein function [25, 26, 37-41]. Kinesin-3 dependent early endosome movement has also been found in *Neurospora crassa* [42].

In *A. nidulans* and *N. crassa*, there is a strong enrichment of proteins involved in endocytosis at a collar right behind the hyphal tip [5, 43-46]. However, despite this preferential localization of the endocytosis machinery close to the hyphal tip, it has been observed using the dye FM4-64 that endocytosis occurs along the hyphae [47]. Presumably the primary endocytic vesicles fuse with Rab5 (RabA and RabB in *A. nidulans*)-positive early endosomes that undergo bidirectional movements [37, 48, 49]. Early-endosome movement driven by dynein is normally associated with early endosome maturation into Rab7 (RabS of *A. nidulans*)-positive late endosomes that are located away from the hyphal tip [49]. However, early endosome maturation into late endosomes, as indicated by the appearance of RabS-positive endosomes, can still occur at the hyphal tip in cells defective in dynein function [49]. This result indicates that the failure of dynein-driven early endosome movement does not inhibit early endosome maturation *per se.* Thus, although endosome maturation is essential for fungal growth and defects in endosome maturation cause severe inhibition in colony growth [48, 49], mutants impaired in dynein-driven early endosome movement can form relatively healthy colonies, making it possible for using them for imaging and biochemical studies. While the functional significance of bi-directional transport is not fully understood, it has been shown recently that RNA molecules, signaling proteins and ribosomes can hitchhike on motile early endosomes to be distributed in hyphae, which may be particularly critical for growth of fungi with relatively long hyphae such as *U. maydis* [50-52].

The direction of early endosome transport is controlled by kinesin-3 and dynein but the detailed mechanisms behind this control may differ in different fungi. In *U. maydis*, the direction seems to depend mainly on whether dynein is bound to the early endosome. While there are multiple (about 4-5) kinesin-3 molecules on an early endosome moving toward the plus end, binding of a single dynein molecule seems sufficient to switch direction [53]. As a result, while kinesin-3 can be seen on early endosomes moving by dynein towards the minus end, dynein has never been seen to be associated with an early endosome that undergoes plus-end-directed movement [53]. Thus, it seems that in *U. maydis*, either dynein always overpowers kinesin-3 or kinesin-3 is turned off during the minus-end-directed movement. In *A. nidulans*, however, dynein on plus-end-directed early endosomes was observed [26], suggesting that either kinesin-3 is capable of overpowering dynein or dynein could also be regulated to be in an inactive state on the same cargo. How these motors are coordinated is a question of general interest in the motor field that needs to be further studied.

Accumulation of dynein at microtubule plus ends is important for early endosome movement

Although dynein is a minus-end-directed motor, it accumulates at microtubule plus ends with a variety of plus-end tracking proteins (+TIPs) including CLIP-170, EB1 and the dynein regulator dynactin [18-20, 54]. Accumulation of GFP-labeled cytoplasmic dynein at both the growing and shrinking microtubule plus ends was first demonstrated in *A. nidulans* where dynein heavy chain molecules form motile comet-like structures near the hyphal tip [24, 55]. In *A. nidulans*, this accumulation depends on the plus-end-directed kinesin-1 [56]. Kinesin-1 is also required for dynein's plus-end accumulation in *U. maydis* and *C. elegans* neurons [25, 57]. The functional significance of the plus-end accumulation of dynein in fungal early endosome movement was first demonstrated in *U. maydis* where majority of early endosomes undergoing plus-end-directed movement were found to switch direction at the microtubule plus-end [25]. Most

significantly, while kinesin-1 is not required for activating dynein ATPase activity [39], loss of kinesin-1 causes early endosomes to abnormally accumulate at the hyphal tip, which is similar to what occurs in mutants defective in dynein function [25, 26, 37-39]. These results suggest that accumulation of dynein molecules at microtubule plus ends might increase the opportunity for an early endosome to interact with a dynein motor. In *U. maydis,* dynein molecules at the plus ends can be seen to move away and meet the early endosomes as they are being transported to the plus end by kinesin-3, thereby reversing the direction of early endosome movement [53]. In addition, a 50% decrease in the plus-end accumulation of dynein led to an increase in the number of early endosomes that fall off the microtubule tracks, suggesting that dynein at the plus end captures early endosomes that are transported to the plus end by kinesin-3 [58].

Mutations in the motor and tail domains of dynein heavy chain affect early endosome movement

Cytoplasmic dynein is a multi-subunit complex that consists of two heavy chain motors (HCs, ~500 kDa each) associated with intermediate chains (ICs, ~74 kDa), light intermediate chains (LICs, 50-60 kDa) and light chains (LCs, 8 kDa, 14 kDa and 22 kDa) [59, 60] (Figure 2). The cytoplasmic dynein we discuss in this review is cytoplasmic dynein 1. However, studies on axonemal dynein and cytoplasmic dynein 2, which is required for intraflagellar transport, have also provided insights into dynein motor mechanism [61, 62]. The dynein heavy chain (HC) contains an N-terminal tail that is required for intra-dynein subunit interactions and a C-terminal motor unit, which contains six AAA (*A*TPase *a*ssociated with cellular *a*ctivities) domains organized into a ring-like structure and the microtubule-binding stalk emerges from AAA4 (Figure 2) [61-67]. AAA1 is the major site of ATP hydrolysis [68], and other AAA sites play regulatory roles [12, 69-73]. The conformational change during the ATPase cycle at the first AAA domain is transmitted to the microtubule-binding stalk to cause the power stroke, which

involves a change in the orientation of the linker located adjacent to the first AAA domain (Figure 2) [61, 62, 65].

Various *A. nidulans* dynein HC mutations were found to affect early endosome movement. In an AAA1 mutant that is defective in ATP hydrolysis, dynein molecules are still enriched at the microtubule plus ends, but early endosomes are blocked at the hyphal tip [39]. Besides driving early endosome movement, cytoplasmic dynein is well known to be important for the migration of nuclei towards the hyphal tip to allow even nuclear distribution of the multiple nuclei along hyphae [74-76]. The mechanism of nuclear distribution in filamentous fungi is not fully understood but appears to involve the role of dynein in regulating the dynamics of microtubules [24, 74, 77-80]. Interestingly, a recent screen for organelle distribution mutants in *A. nidulans* has identified two dynein HC mutations, in AAA1 and AAA3 respectively, which are more detrimental to early endosome migration than to nuclear migration [41]. Since analogous mutations in budding yeast dynein HC cause a significant reduction in the speed of dynein movement, these results indicate that a normal level of dynein motor activity is more crucial for early endosome movement than for nuclear migration [41]. In a different screen, a HC tail mutation was found to be important for both early endosome movement and nuclear distribution but did not seem to affect dynein complex assembly or dynein-dynactin interaction [40]. While the mechanism of this tail mutation is unclear, the importance of the dynein tail in dynein motor function has been recognized in other systems as well [81-86]. Given that the HC motor domains may be regulated and given the recent model that dynactin and cargo-adaptors bound to the dynein tail may modulate the function of the motor domain [87][183], it seems possible that the tail mutation may affect the proper tail-mediated modulation of dynein motor function.

The dynactin complex and its role in early endosome transport

The dynactin complex is important for almost all of the in vivo functions of cytoplasmic dynein [11]. It was initially discovered as a complex required for in vitro vesicle transport by dynein, and its major subunit p150 was identified as a dynein-associated peptide homologous to the Drosophila Glued protein (Hence this subunit was named $p150^{Glued}$) [88-90]. As demonstrated by in vitro experiments, $p150^{Glued}$ (p150 here) binds directly to the dynein IC (Figure 2) [91-93]. The structure of the vertebrate dynactin complex has been studied [94-96] [183]. Within the dynactin complex, eight Arp1 (actin-related protein 1) subunits and one beta-actin form an actin-like mini-filament of 37 nm [96] [182-183] (Figure 2). The $p150^{Glued}$ subunit, dynamitin (p50) and the p24 subunit form a shoulder/sidearm complex [11]. One end of the Arp1 filament is capped by the barbed-end capping proteins, and the other end binds to the pointed-end complex consisting of an actin-related protein Arp11, p62 and two other small subunits, p25 and p27 [97]. In *A. nidulans*, loss of Arp11 or p62 significantly reduces the amount of Arp1 as judged by p150-pull-down assays, and a similar result was also obtained in mammalian cells, suggesting that these two pointed-end proteins are important for the integrity of the dynactin complex [98, 99].

Dynactin has been implicated in targeting dynein to microtubule plus ends, in recruiting dynein to membranous cargoes and in enhancing dynein processivity (the ability to move along a microtubule for a long distance without falling off the track) [55, 98, 100-106]. Purified vertebrate dynactin enhances dynein processivity in vitro and the N-terminal microtubule-binding domain of p150 is implicated in this function [101, 107, 108]. In addition, dynein processivity is also supported by other mechanisms [109, 110]. Purified mammalian dynein is much less processive than yeast dynein in single-molecule motility assays in vitro, and adding dynactin and BICD2, a cargo adapter that enhances dynein-dynactin interaction, dramatically enhances dynein processivity [111, 112]. The dynactin complex has also been implicated in recruiting dynein to membranous cargoes, and the physical interaction between Arp1 and beta III spectrin provided the initial support for this notion [113, 114]. However, current data suggest that dynein may be

recruited to membranous cargoes via different mechanisms, some of which involve dynactin but others involve the direct binding between a cargo adapter and a component of the dynein complex [22, 98, 100, 105, 115-120].

In mammalian cells and filamentous fungi, dynactin is critical for dynein-mediated early endosome movement [25, 36]. In filamentous fungi, dynactin plays at least two different roles. First, it is required for the plus-end accumulation of dynein [55, 56], and second, it is required for the physical interaction between dynein and early endosomes [105]. One important role of dynactin is in promoting the accumulation of dynein at the microtubule plus end, a notion supported by evidence in *A. nidulans*, *U. maydis* and mammalian cells [25, 26, 55, 56, 58, 99, 103, 104]. In *A. nidulans,* this function of dynactin requires the microtubule-binding ability of p150 [104]. Dynactin itself is also found to accumulate at the microtubule plus ends in cultured cells of higher eukaryotes and in fungal hyphae. This localization is relevant for the loading of dynein cargoes [54, 121]. In *A. nidulans*, the plus-end localization of dynactin, like that of dynein, also requires kinesin-1 [56, 104]. In addition, the microtubule-binding ability of p150 is critical for the plus-end accumulation of dynactin itself [104]. It is not clear whether dynein and dynactin are transported by kinesin-1 toward the plus end as a complex or if they are transported separately. However, it is likely that dynactin enhances the interaction between dynein and microtubules, thereby facilitating its transport towards the plus end by kinesin-1.

In *A. nidulans*, dynactin is also required for the interaction between dynein and early endosomes, with crucial involvement of the p25 subunit [105]. p25 is not present in yeasts, where dynein has not been implicated in moving vesicles [97, 122]. As first shown in *N. crassa*, p25 is not required for dynein-mediated nuclear migration, a process that requires other core dynactin components, but it is required for vesicle transport [123, 124]. In filamentous fungi and mammalian cells, p25 is only required for a subset of dynactin functions including early endosome transport [98, 105, 123]. In *A. nidulans*, either loss of p25 or Arp1 downregulation

causes marked reduction in the dynein-early endosome interaction [105]. Because the integrity of Arp1 in the dynactin complex is not obviously affected by loss of p25, the effect of p25 loss on the dynein-early endosome interaction appears specific. This idea is consistent with the results obtained from mammalian cells where the p25/p27 subunits are not critical for the overall integrity of the dynactin complex but are required for enhancing the physical interaction between dynein and membranous cargoes [98].

LIS1 and its role in early endosome transport

LIS1 is a WD-repeat-containing protein that regulates dynein function mainly by causing dynein to bind tightly to microtubules, without affecting dynein's ATPase activity [125-128]. One interesting difference between dynactin and LIS1 is that LIS1 but not dynactin is present in cilia/flagella, and thus, LIS1 may be a ubiquitous dynein regulator that regulates the function of not only cytoplasmic dynein but also axonemal dynein [129, 130]. The Lis1 gene was originally cloned as a causal gene for lissencephaly, a human brain developmental disorder characterized by defective neuronal migration [131]. In *A. nidulans*, loss-of-function mutations of the LIS1 homolog NudF were found to cause the same nuclear-distribution defect as that caused by dynein deficiency, and NudF was placed in the dynein pathway by double mutant analysis [132, 133]. The LIS1 homolog Pac1 in the budding yeast was also found to be a protein in the dynein pathway [134]. Pac1 is required for dynein localization to the microtubule plus ends [135, 136]. The physical interactions and the functional connection between LIS1 and dynein have been detected in higher eukaryotes also [137-142]. While AAA1 was initially thought to be the binding site for LIS1 in mammalian cells [143], recent EM studies showed convincingly that Pac1/Lis1 binds to AAA4, which is located next to the stalk that leads to the microtubule-binding domain [125, 144]. Interestingly, although genetic data indicate that Lis1 plays a positive role in dynein function, neither mammalian LIS1 nor Pac1/Lis1 functions as a dynein activator in vitro, but rather clamps dynein on microtubules and stops it from moving in vitro [125, 127].

Mechanistically, this inhibitory effect could be caused by Pac1/Lis1 occupying the site on dynein where the linker, an important mechanical element of dynein, normally moves to during the mechanic cycle of dynein [144], and presumably, without the proper movement of the linker, the dynein-microtubule interaction is maintained at a high-affinity state.

LIS1 interacts with the coiled-coil protein NudE (Ro11 *in N. crassa*), which was identified as a multi-copy suppressor of a *nudF* mutant in *A. nidulans* [145, 146]. In higher eukaryotes, NudE and the NudE-like protein Nudel contain dynein-binding sites, and the N-terminus of dynein intermediate chain implicated in binding p150 is also involved in binding NudE/Nudel [147-152]. NudE/Nudel participate in dynein function by recruiting LIS1 to dynein, and together, NudE/Nudel and LIS1 are able to enhance dynein motor function under heavy-load conditions [126]. Experimental evidence also suggested that NudE/Nudel relieves dynein inhibition in an in vitro microtubule-sliding assay [127], but this notion does not seem to be consistent with data in *A. nidulans*, *S. cerevisiae* and Xenopus egg extracts where overexpression of LIS1 homologs suppress the defect caused by loss of NudE [145, 152, 153]. In the case of *S. cerevisiae* dynein, NudE is required for recruiting LIS1 to dynein but does not play a role in relieving Pac1/LIS1 inhibition in vitro [125]. Interestingly, recent work also implicated the dynactin complex and the small GTPase Rab6 in relieving LIS1 inhibition, and showed that loss-of LIS1 negatively affect dynein-dynactin interaction, suggesting that these dynein regulators may have interconnected functions [152, 154].

In *U. maydis* and *A. nidulans*, loss of LIS1/NudF causes a defect in dynein-mediated early endosome transport, indicating that LIS1 is positively required for early endosome movement. However, in *A. nidulans* loss of NudF/LIS1 does not have any apparent effect on dynein ATPase activity [39], and dynein molecules can be seen to move with a normal speed in live hyphae of a Δ*nudF* mutant [26]. Nevertheless, dynein's interaction with microtubules is weakened upon down-regulation of NudF expression [39], consistent with the in vitro data that

LIS1 enhances dynein-microtubule interaction [125-127]. It is likely that the LIS1-mediated enhancement of the dynein-microtubule interaction is a major function of LIS1 in early endosome movement. Just like dynein and dynactin, NudF/LIS1 itself also accumulates at microtubule plus ends, and the +TIP ClipA (Clip-170 in *A. nidulans*) and NudE play a redundant role in this accumulation [27]. Interestingly, in both *U. maydis* and *A. nidulans*, unlike dynein and dynactin, which are associated with early endosomes moving towards the minus-end, LIS1 was only seen to associate with a very low percentage of early endosomes that are moving with dynein, as if LIS1 is released from the early endosome (and presumably off the dynein complex) very soon after the minus-end-directed movement starts [25, 26]. In *A. nidulans*, while there is a significant decrease in the frequency of dynein-mediated early endosome transport in the Δ*nudF* mutant, the speed of transport is not significantly different from that in wild type cells [26]. Based on these results, it has been proposed that LIS1 functions as an initiation factor for dynein-mediated early endosome movement [26].

Exactly how LIS1 helps dynein to initiate the movements of early endosomes in filamentous fungi still requires further study. In comparison, the mechanism of LIS1 action in *S. cerevisiae* is better understood because Pac1/LIS1 is clearly required for the microtubule-plus end accumulation of dynein, and as plus-end dynein gets delivered to the cell cortex, where it can be anchored to pull astral microtubules, loss of Pac1 prevents dynein from being targeted to the site of its action [135, 136, 155, 156]. However, LIS1 does not seem to be required for the microtubule plus-end accumulation of dynein in *A. nidulans* or *U. maydis* as plus-end dynein comets are present upon loss of LIS1 [25, 26, 39, 56]. Upon loss of NudF/LIS1 in *A. nidulans*, both plus-end comets and a cloud of GFP-dynein appear at the hyphal tip, which was originally interpreted as dynein falling off the microtubule ends [56]. Later, because early endosomes were also seen to accumulate at the hyphal tip, this hyphal-tip cloud of GFP-dynein was interpreted as dynein-endosome co-localization [39]. However, results from a more recent study

indicate that the dynein cloud present at the hyphal tip of Δ*nudF*/Lis1 hyphae is unlikely to represent co-localization between dynein and early endosomes, suggesting that NudF/Lis1 may be required for dynein-early endosome interaction [26]. Further experiments will be needed to determine if loss of LIS1 results in the failure of dynein to physically bind its cargo at the microtubule plus end or the failure of cargo-bound dynein to initiate its movement without falling off the microtubule track.

LIS1 in non-polarized mammalian cells does play an important role in the plus-end accumulation of dynein, which is similar to what happens in the budding yeast but differs from the situation in filamentous fungi [103]. However, it should also be noted that similar to the situation in filamentous fungi but in contrast to that in budding yeast, the plus-end localization of dynein in mammalian cells also requires dynactin [103]. In highly polarized neurons, LIS1 is not only important for the initiation of vesicle movement from the distal region but also important for movement in the middle region, which differs from the roles of the +TIP Clip-170 and the microtubule-binding region of p150 dynactin, which are only required for transport initiation at the distal region [157]. It should also be pointed out that in some cell types LIS1 seems to be only important for moving heavy cargoes but in other systems LIS1 is also required for moving small ones [157-162]. Despite these discrepancies, it has been well agreed that LIS1 is positively required for enhancing dynein-microtubule interaction both in vitro and in vivo, which may be the key for explaining the requirement for LIS1 in different experimental systems.

Function of Hook in dynein-mediated early endosome transport

Recently, two fungal Hook proteins, HookA in *A. nidulans* and Hok1 in *U. maydis*, have been found to serve as early endosomal adapters for dynein [13, 15]. In addition, Hok1 has also been implicated in recruiting kinesin-3 to early endosomes, suggesting that Hook may play a role as a scaffold protein that co-ordinates the functions of dynein and kinesin-3 motors on the same

cargo [13, 22]. The prototype Hook protein was initially identified in Drosophila, and it is required for the proper formation or stabilization of multivesicular bodies in the endocytic pathway [163-165]. There are three Hook proteins in mammalian cells, Hook1, Hook2 and Hook3, which play different cellular roles. Hook1 is involved in sperm head morphogenesis [166], and it is required for endosome sorting of non-clathrin cargo in a microtubule-dependent fashion [167]. Hook2 is involved in centrosome function, aggresome formation and primary cilium morphogenesis [168-170], and Hook3 is associated with Golgi [171]. All the Hook proteins contain an N-terminal domain implicated in microtubule-binding, an extended central coiled-coil domain implicated in homodimerization, and a divergent C-terminal domain implicated in organelle association [171, 172]. Besides the three hook proteins, a novel Hook-Related Protein (HkRP) family has also been identified. These HkRPs have a domain organization similar to the Hook proteins, but they are larger in size [173]. Overexpression of C-terminal domain of HkRP1 affects distribution of the early endosome marker sorting-nexin 1 but not the early endosome antigen-1 (EEA1), suggesting that HkRP1 may only affect the tubulation of early endosome subdomains [173]. Interestingly, the *C. elegans* Hook homolog Zyg-12 has been implicated in linking dynein to nuclear membrane to ensure the connection between the centrosome and the nucleus [174].

Both HookA in *A. nidulans* and Hok1 in *U. maydis* were identified in genetic screens for mutants defective in early endosome distribution. In *A. nidulans*, this screen was aimed at mutants that are specifically defective in dynein-early endosome interaction, and thus, the selection of the original *hookA* mutant was based on the normal dynein-localization to the microtubule plus-ends and dynein-mediated nuclear distribution in the mutant [15]. Both HookA and Hok1 associate with early endosomes, and this association depends on their respective C-terminal cargo-binding sites [13, 15]. As shown by pull-down assays, HookA interacts with dynein-dynactin. However, the interaction between microtubules and HookA in the absence of bound dynein-dynactin was undetectable [15]. The *A. nidulans* HookA-dynein-dynactin interaction depends on

the N-terminal part of HookA as evidenced by pull-down assays [15], and live cell imaging in *U. maydis* showed that the N-terminal part of Hok1 free from early endosomes can co-migrate with dynein in vivo [13]. Upon loss of HookA or Hok1, dynein-early endosome interaction is significantly weakened as judged by pull-down assays in *A. nidulans* or by cell imaging of *U. maydis* [13, 15]. These results support the idea that Hook proteins serve as early endosome adapters for dynein. The physical interaction between Hook and dynein-dynactin has also been shown subsequently in mammalian cells [111]. Recently, it has been shown that while isolated mammalian dynein is almost completely non-mobile in single-molecule assays, adding cargo adapters such as BICD2 significantly enhances dynein-processivity, and Hook3 has also been implicated in a similar function [111]. Thus, it is possible that besides serving as cargo adapter, Hook proteins may also be involved in activating dynein as in the case of BICD2 [111]. However, it should be pointed out that in *U. maydis*, dynein molecules can be seen to move away processively from the microtubule plus ends in the absence of Hok1 [13], arguing against the idea that fungal Hook proteins are required for activating the dynein motor.

U. maydis Hok1 is also important for recruiting kinesin-3, but without Hok1, there is still one kinesin-3 molecule on early endosome, which might be able to move early endosomes in the ΔHok1 mutant to the hyphal tip [13]. In wild type hyphae, when an early endosome reaches the microtubule plus end at the hyphal tip, the bound kinesin-3 molecules seem to detach from it right before dynein binds to this early endosome [13], which then recruits more kinesin-3 molecules during dynein-mediated movement [13]. Whether Hook is involved in this transient release of kinesin-3 before dynein binding deserves further study.

Exactly how Hook interacts with dynein-dynactin remains an important question. Based on yeast two-hybrid data it was initially proposed that the putative microtubule-binding domain at the N-terminus of the *C. elegans* Hook homolog, Zyg-12, binds to a dynein light intermediate chain [174], but this two-hybrid interaction has never been confirmed by biochemical data. Our

biochemical experiments showed that HookA without its C-terminal early endosome-binding region pulls down both dynein and dynactin, and while dynein is required for HookA to pull-down dynactin, dynactin is also required for HookA to pull-down dynein [15]. Thus, only the tripartite super-complex involving HookA-dynein-dynactin is stably formed. Importantly, the HookA-dynein-dynactin interaction requires p25, a component of dynactin whose absence does not significantly affect the overall integrity of dynactin, suggesting that p25 is specifically required for the formation of the tripartite complex [15]. However, whether p25 directly binds HookA is still unclear.

It has been shown previously that all three human Hook proteins have been found as components of the FTS/Hook/FHIP (FHF) complex, which contains two additional proteins, Fused Toes (FTS), a variant E2 ubiquitin-conjugating enzyme domain-containing protein and the FTS- and Hook-Interacting Protein called FHIP [172]. The FHF complex was initially discovered during the search for FTS-binding proteins, and it has been found that the C-termini of Hook1 and Hook3 interact with FTS [172]. The FHF complex interacts with the components of the homotypic vesicular protein-sorting (HOPS) complex, which is recruited to late endosomes [172]. In *U. maydis*, Hok1 physically associates with the FTS and FHIP proteins in pull-down assays, indicating that the FHF complex is conserved from lower to higher eukaryotic systems [13]. In *A. nidulans*, the genetic screen on early-endosome distribution mutants that identified the HookA gene also identified the gene encoding FhipA (FHIP in *A. nidulans*) [14]. Functions of FhipA and FtsA (FTS in *A. nidulans*) have been studied using deletion mutants of these genes, and loss of either FhipA or FtsA causes a defect in early endosome distribution, although the loss of FhipA produces a more severe defect than that produced by FtsA based on the percentage of hyphal tips with an abnormal accumulation of early endosomes [14]. Imaging analyses suggest that HookA-early endosome association requires FhipA and FtsA, and that FtsA-early endosome association also requires FhipA and HookA [14]. However, FhipA-early

endosome association occurs in the absence of HookA or FtsA, although the protein level of FhipA is significantly decreased in the absence of these partners [14]. Thus, while the formation of the FHIP-Hook-FTS complex is important for Hook to interact with early endosomes, FHIP appears to be more directly linked to early endosomes than either FTS or Hook.

Exactly how the FHF complex interacts with early endosomes is unclear, and since FHIP does not contain any membrane-spanning domain, it is likely that other proteins on early endosomes are required for its early-endosome interaction. RabB, an ortholog of Rab5, and some of its recruited proteins, are likely to be required. Rab GTPases have been implicated in recruiting motor proteins to organelles; for example, Rab7 is important for recruiting dynein to late endosomes/lysosomes [100, 118, 119]. Rab5 is an early-endosome-specific Rab GTPase [175, 176], and the Drosophila Hook has recently been implicated as a Rab5 effector [177]. In *A. nidulans*, there are two Rab5 paralogs, RabA and RabB, both of which localize to early endosomes [48]. Interestingly, while early endosomes move normally in the Δ*rabA* mutant, early endosomes are completely static in the Δ*rabB* mutant [48], suggesting a role of RabB in motor recruitment. RabB (Rab5) recruits to early endosome the CORVET complex (CORVET: Rab5 effector class C core vacuole/endosome-tethering) [48, 178]. This complex is important for early endosome fusion, which serves to increase the surface area of early endosomes to facilitate formation of late endosomes/multivesicular bodies [179]. CORVET contains six Vps proteins of which four are also components of the homotypic vesicular protein-sorting (HOPS) complex on late endosomes [48, 178]. Interestingly, the human FTS-Hook-FHIP complex interacts with Vps18, a component shared by CORVET and HOPS [172], suggesting that a similar component in fungi may recruit the FTS-Hook-FHIP complex to early endosomes.

Conclusions

Early endosomes are a major class of cytoplasmic dynein cargoes in many cell types. Their robust movements in filamentous fungi have made these organisms ideally suited for studying their transport mechanisms. Our current knowledge of fungal early endosome motility has been mainly gained from studies in *A. nidulans* and *U. maydis*. In both systems, dynein and kinesin-3 are the early endosome-bound motors that power the bi-directional movements of these organelles. Cytoplasmic dynein accumulates at the microtubule plus ends near the hyphal tip, an early endosome-loading site for minus-end-directed transport, and the plus-end accumulation of dynein requires kinesin-1 as well as the dynactin complex. The dynactin complex plays at least two important roles in dynein-mediated early endosome transport: 1) the plus-end accumulation of dynein and 2) the physical interaction between dynein and early endosomes. Specifically, the 150 subunit of dynactin, especially its N-terminal microtubule-binding site, is important for dynein to accumulate at the microtubule plus end, and the p25 protein of dynactin is required for the physical interaction between dynein and early endosomes. The dynein-binding protein LIS1 is also critical for dynein-mediated early endosome movement in both *U. maydis* and *A. nidulans* and its mechanism still needs further study. An interesting observation is that unlike dynactin, which remains associated with dynein-driven early endosomes, LIS1 does not co-localize with these moving early endosomes. Thus, LIS1 is unlikely to be required for maintaining the interaction between dynein and early endosomes or the motility of dynein-bound early endosomes after movement has started. Finally, the Hook-FTS-FHIP complex has been found to mediate the physical interaction between dynein-dynactin and early endosomes. These proteins are associated with early endosomes independently of dynein. Hook is responsible for interacting with dynein-dynactin, and p25 is required for this interaction. However, it is not clear whether this interaction is direct or mediated by other unknown components. FTS and FHIP associate with the C-terminal cargo-binding site of Hook,

and they are both required for Hook-early endosome interaction. It appears that FHIP is more directly bound to early endosome than FTS and HOOK, but how it interacts with early endosomes still needs to be defined.

In summary, recent work in filamentous fungi has identified the basic machinery for dynein-mediated early endosome movement, but important questions remain. Filamentous fungi are, in general, not only excellent genetic systems but also well suited for live cell imagining of membrane trafficking, and in addition, their rapid growth facilitates biochemical analysis. With the availability of fungal genome sequences and newly developed tools for genome manipulation [180, 181], insights on the detailed mechanism of early endosome motility should continue to emerge from studies using filamentous fungi as experimental systems.

Figure legends:

Figure 1 A schematic diagram showing microtubule organization in multinucleated fungi such as *A. nidulans.* Blue circles: nuclei. Blue lines: microtubules. Red circles: Spindle-Pole Bodies. A microtubule plus end is labeled as "+" and minus end as "-". In the middle of hyphae, microtubules are of mixed polarity, but in a region close to the hyphal tip, the microtubule plus ends face the hyphal apex.

Figure 2 Schematic diagrams of the cytoplasmic dynein complex and the dynactin complex. (A) The dynein complex. This diagram was modified from [59]. The dynein heavy chain motor (HC) and other subunits, including the intermediate chain (IC), light intermediate chain (LIC) and three families of light chains (Rob1, Tctex1, LC8) are shown. (B) Different domains of the dynein heavy chain. This diagram was modified from [66]. Each heavy chain (HC) contains the N-terminal tail and the C-terminal motor unit with six AAA domains (marked 1-6) that are organized in a ring-like structure. The linker is an important mechanical element connected to AAA1. The coiled-coil stalk extends out from AAA4 and leads to the microtubule-binding domain. The buttress is a coiled-coil hairpin that extends out of AAA5 and contacts the stalk. (C) A simplified diagram of dynein-dynactin when the two complexes are together. The dynactin complex is a modified from a previous publication [11]. The interaction between the dynein IC and p150 dynactin is shown, but the recently identified interaction between the HC tail and Arp1 is not drawn in this diagram [183].

Figure 3 Hook-dynein-dynactin interaction requires the N-terminal region but not the C-terminal cargo-binding domain of Hook. Here we present data obtained from *A. nidulans* Hook (HookA) to show this point. (Left) A diagram of HookA, ΔC-HookA, ΔC1-HookA and ΔN-HookA. Red box: N-terminal region; Blue boxes: coiled-coil domains; Brown box: the C-terminal domain. The functions of the N- and C-terminus are indicated at the bottom. Note that in the ΔC1 mutant, 13

aa at the end of the third coiled-coil are deleted, which disrupts the function of the C-terminal domain in early endosome binding. (Right) The dynein HC, the p150 subunit of dynactin and NudF/LIS1 (a dynein-binding protein) were pulled down with HookA-GFP, which requires the N-terminus but not the C-terminus of HookA. This figure was modified from [15].

Figure 4 Model showing the FTS-Hook-FHIP complex linking dynein-dynactin to early endosomes. Hook (blue, depicted as a dimer [172]) interacts with dynein-dynactin complexes [13, 15], but the mechanism of the interaction is not clear (indicated by "??"). The C-terminus of Hook interacts with FTS (brown) [172]. FHIP (red) is depicted to be most close to early endosome [14]. Missing linkers are indicated by "??".

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Figure 1

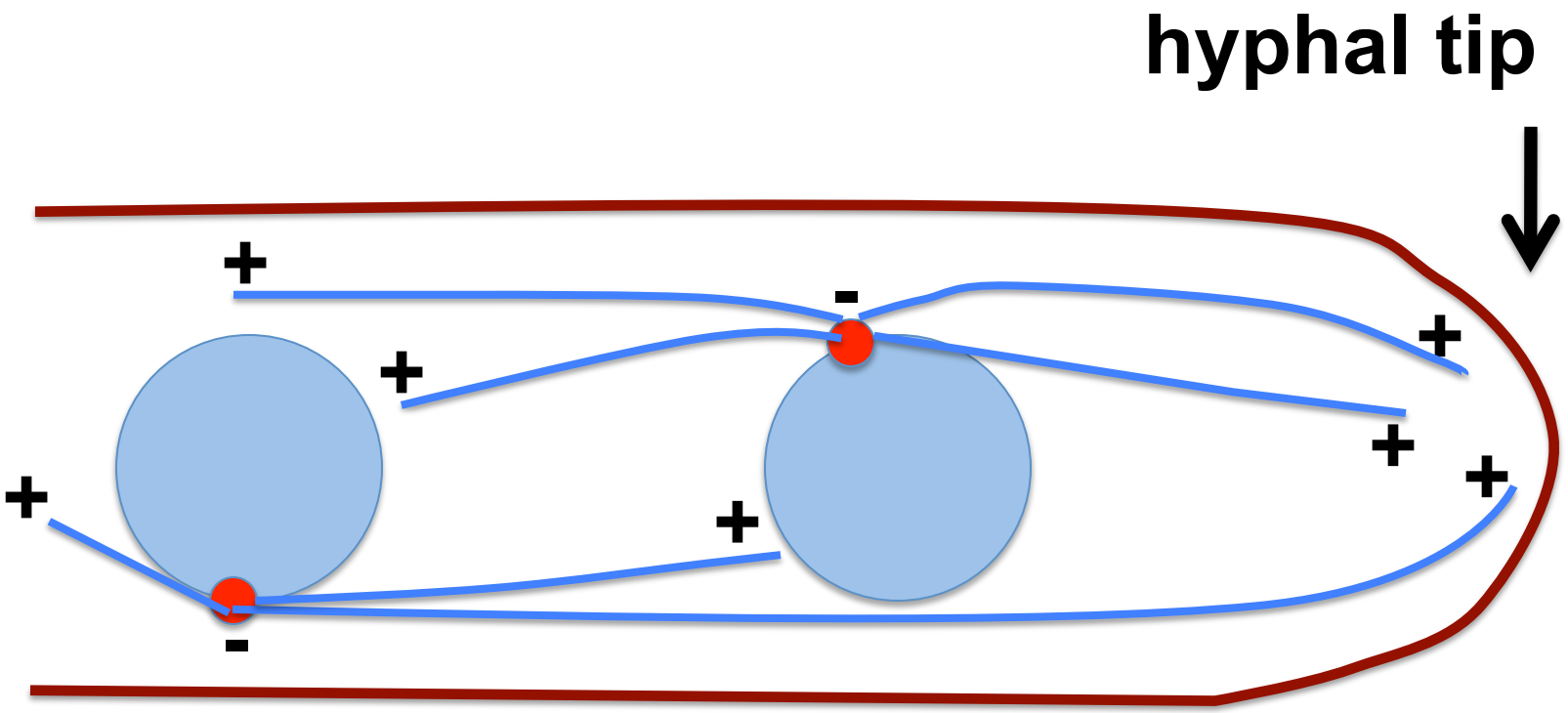


Figure 2

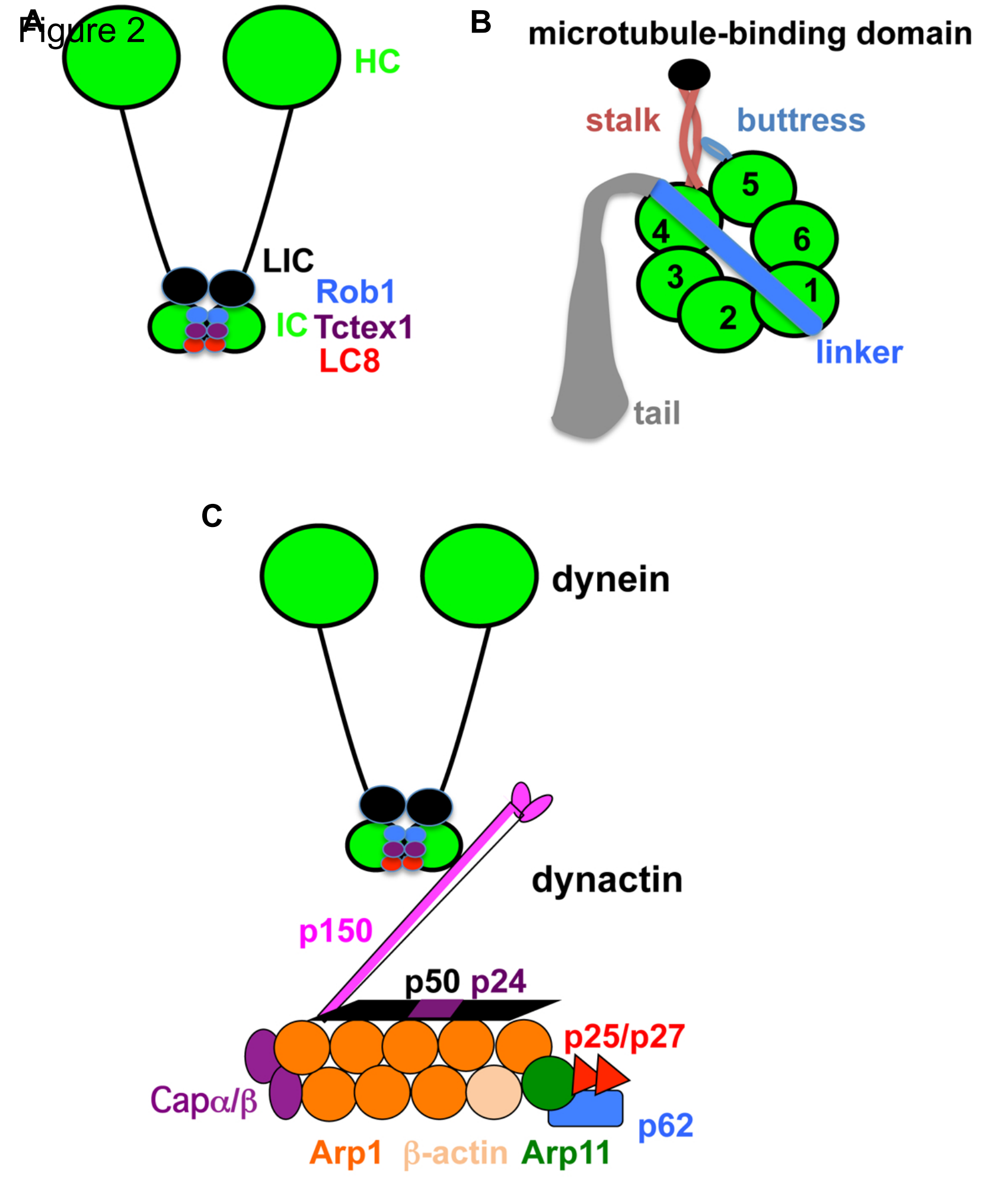
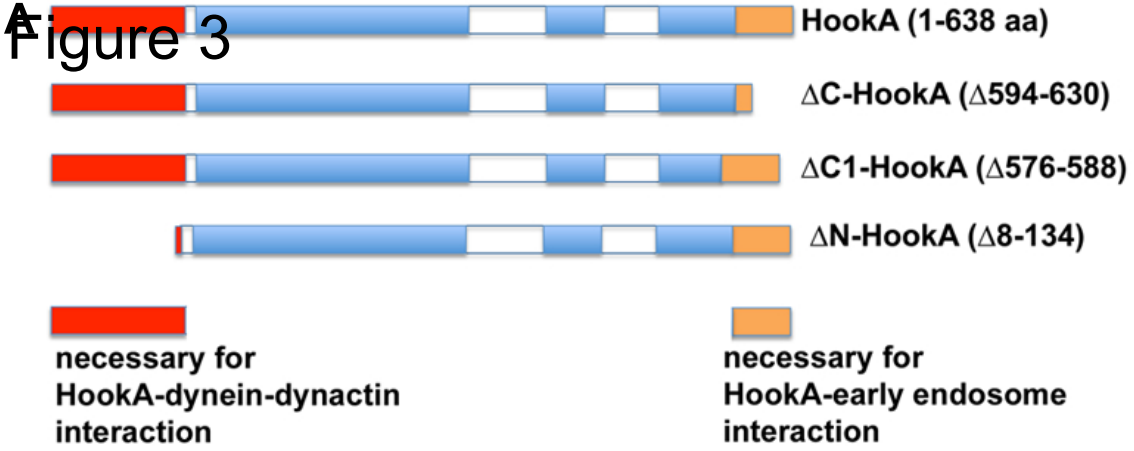

A
HC
LIC
Rob1
IC Tctex1
LC8
B
microtubule-binding domain
stalk
buttress
5
4
6
3
1
2
linker
tail
C
dynein
dynactin
p150
p50 p24
p25/p27
Capα/β
p62
Arp1 β-actin Arp11

Figure 3


A
HookA (1-638 aa)
ΔC-HookA (Δ594-630)
ΔC1-HookA (Δ576-588)
ΔN-HookA (Δ8-134)
necessary for HookA-dynein-dynactin interaction
necessary for HookA-early endosome interaction

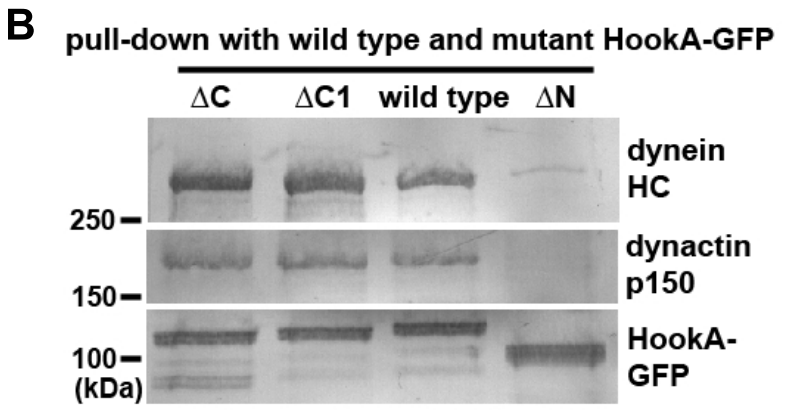
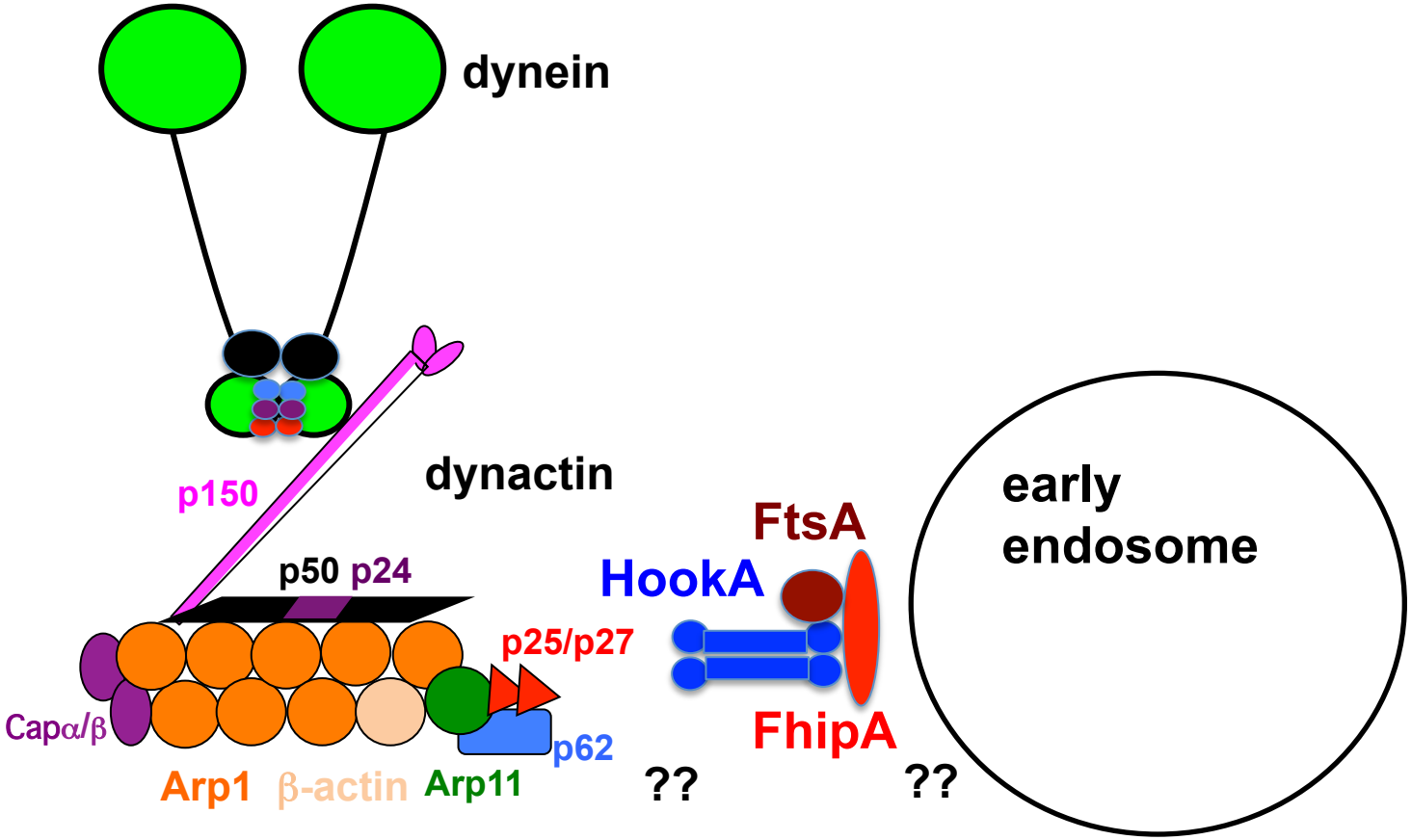

B
pull-down with wild type and mutant HookA-GFP
ΔC
ΔC1
wild type
ΔN
250
150
100
(kDa)
dynein HC
dynactin p150
HookA-GFP

Figure 4


dynein
dynactin
p150
p50 p24
p25/p27
p62
Capα/β
Arp1
β-actin
Arp11
??
FtsA
HookA
FhipA
??
early
endosome