Published in final edited form as:

Bioessays. 2013 August ; 35(8): 677–682. doi:10.1002/bies.201300016.

Stopped in its tracks: negative regulation of the dynein motor by the yeast protein She1

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Summary

How do cells direct the microtubule motor protein dynein to move cellular components to the right place at the right time? Recent studies in budding yeast shed light on a new mechanism for directing dynein, involving the protein She1. She1 restricts where and when dynein moves the nucleus and mitotic spindle. Experiments with purified proteins show that She1 binds to microtubules and inhibits dynein by stalling the motor on its track. Here I describe what we have learned so far about She1, based on a combination of genetic, cell biology, and biophysical approaches. These findings set the stage for further interrogation of the She1 mechanism, and raise the question of whether similar mechanisms exist in other species.

Introduction

How do cells organize their cytoplasm? The cell is a dark and crowded place where the arrangement of contents is critical for homeostasis. The microtubule network provides a framework for organization. Microtubules are dynamic linear polymers that emanate from tightly controlled nucleation sites – centrosomes, basal bodies, and Golgi – and extend to distant regions of the cell. These extensions provide tracks for motor proteins to power transport or transmit force. Networks of microtubules and motor proteins support diverse processes, including organelle transport, ciliary and flagellar beating, and the separation of the duplicated genome during mitosis.

The microtubule motor dynein is a multi-protein complex that is employed for many processes across species. The catalytic subunit is dynein heavy chain, an immense protein (>500kD) that includes a ring of AAA+ (ATPase Associated with diverse cellular Activities) ATPase domains coupled to a microtubule binding stalk and swinging linker arm. The mechanism of dynein motility has been elusive due to the size of the heavy chain, and historical lack of a suitable recombinant system, until the development of yeast and *Dichtystelium* models [1, 2]. The current model is based on a synergy of data from structures of heavy chain fragments, cryo-EM images of axonemal dyneins, and in vitro analysis of the behavior of motile dynein at the single molecule level (for a thorough review, see [3]). In the nucleotide-free (apo) state, dynein binds tightly to the microtubule. ATP-binding to the most N-terminal ATPase domain (AAA1) elicits a conformational rearrangement that is thought to propagate through the ATPase ring to the microtubule-binding domain (MTBD), disrupting its affinity for the microtubule [4–7]. With the MTBD dislodged, the linker arm swings the ATPase ring and MTBD forward [8, 9]. At this point the MTBD is thought to engage in electrostatic interactions with negatively-charged tubulin

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I have no conflict of interest to declare.

tails, promoting a diffusive search for the next tight-binding site [10, 11]. After ATP hydrolysis, microtubule-binding stimulates phosphate release and the productive powerstroke, followed by restoration of the high-affinity MTBD-tubulin interaction. In cells, cytoplasmic dynein heavy chain acts as a dimer; this coupling is necessary for processive movement along microtubules [1]. Thus the nucleotide hydrolysis cycle is coupled with microtubule binding and movement.

Dynein is distinct from the kinesin family of microtubule motors in several important ways. Whereas dynein heavy chain propagates conformational changes over large distances (25nm from AAA1 to MTBD), kinesins are relatively compact: ATPase and MTBD are contained in a single domain [12]. During motility, dynein takes predominantly minus end-directed steps, but often steps ‘off-axis’ and can reverse direction [1, 10, 11, 13, 14]. This may allow dynein to respond to load or navigate crowded microtubule tracks. In contrast, kinesins are typically unidirectional motors that walk a linear path, rarely stepping off-axis [11, 15–17]. Finally, whereas distinct kinesin isoforms have been derived through evolution for specific tasks, a single cytoplasmic dynein heavy chain isoform is used for diverse tasks [18]. This all hints at intrinsic plasticity in dynein; a virtue that may allow the motor to be ‘tuned’ for different modes of transport or force generation.

How do cells harness dynein activity?

Extrinsic regulators, either positive or negative, play a critical role in directing dynein activity in cells. At the molecular level, a positive regulator may promote activity by stabilizing the dynein complex, recruiting dynein to the microtubule, stabilizing a dynein-cargo complex, promoting dynein motility (e.g. velocity, processivity, force generation), or facilitating interactions with other positive regulators. A negative regulator might inhibit any of these. In cells, positive or negative regulators may be defined by their mutant phenotypes; mutants disrupting positive regulators will exhibit a loss-of-dynein phenotype, while those disrupting negative regulators might exhibit hyperactive dynein.

The budding yeast *Saccharomyces cerevisiae* has been a valuable model for defining a genetic pathway of dynein function. In these cells, dynein delivers a genome and nucleus to the nascent daughter cell (the bud) by pulling on astral microtubules from the bud cortex (Fig. 1; reviewed in [19]). Astral microtubules emanate from the cytoplasmic face of the Spindle Pole Body (SPB; yeast centrosome equivalent), which is embedded in the nuclear envelope and nucleates spindle microtubules from its nuclear face. Null mutations in dynein heavy chain are not lethal; however, they impair movement of the mitotic spindle and associated nucleus into the bud [20, 21]. Under these conditions, cells rely on compensatory processes that align the spindle along the mother-bud axis and elongate the spindle to drive one end into the bud [22–24]. Thus, a loss-of-dynein phenotype is defined by two characteristics - ‘lagging spindles’ that enter mitosis while in the mother compartment, and lethality when combined with mutations in compensatory pathways. These phenotypes have been used to identify a number of positive regulators; i.e. genes necessary for dynein function. Many are conserved across species, including the chains of the dynein complex, the multi-protein dynactin complex, LIS1/ Pac1, NudEL/Ndl1, CLIP170/Bik1, and kinesin/ Kip2. The current model for dynein function in yeast is depicted in Fig.1.

Defining negative regulators of dynein has been a greater challenge. Null mutants in negative regulators should increase dynein activity, disrupting control of where and when dynein moves the spindle and nucleus. Dynein activity must be concentrated toward the bud in order to pull an SPB from the mother compartment into the bud. This is facilitated in part by loading dynein and dynactin on astral microtubules that enter the bud [25, 26]. Although the mechanism of selective dynein recruitment to bud-ward microtubules is not clear, it

requires proteins associated with the bud neck, suggesting a signaling pathway based on microtubule-neck interactions [25]. Dynein is also temporally regulated. Dynein-dependent movement is diminished during the G1 and S-phases of the cell cycle, and increased at mitotic onset [22, 24, 27]. Mutants in *KAR9* and DNA-damage checkpoint genes exhibit premature dynein activity, resulting in frequent spindle movement prior to mitosis [24, 28, 29]. The implication of the DNA-damage checkpoint suggests that dynein activity is controlled by transition into mitosis; but the mechanism is not understood.

She1: a novel dynein regulator

She1 was first identified as a dynein regulator based on its null mutant phenotype - excessive spindle movement^{*}. Woodruff and colleagues observed spindle movement prior to mitosis in *she1Δ* mutants, with the spindle often oscillating back-and-forth between the mother and bud [30]. During these movements, astral microtubules slide along the cell cortex in front of the advancing spindle; reminiscent of how cortical dynein pulls astral microtubules. A key feature of these experiments is that they were conducted in cells treated with hydroxyurea, which induces cell cycle arrest in S-phase by depleting dNTPs and stalling replication forks. This prevents entry into mitosis and spindle elongation; therefore, spindle movement in *she1Δ* mutants must be driven by forces on astral microtubules. Consistent with this, *she1Δ* mutants exhibit astral microtubules that detach from the SPB and slide along the cortex [31]. These phenotypes are suppressed by knocking out dynein in the *she1Δ* mutant background [30, 31]. Furthermore, She1 overexpression leads to lagging mitotic spindles at a level equivalent to dynein null mutants [31, 32]. Together, these results indicate that She1 negatively regulates dynein.

Strong evidence suggests that She1 regulates dynein by inhibiting the recruitment of its obligate positive regulator, dynactin. Dynein recruits dynactin to astral microtubule plus ends in a cell cycle dependent manner [26]. Dynactin is most abundant during mitosis, coincident with the timing of dynein activity [26, 30]. This suggests that dynactin recruitment may be rate-limiting in the dynein mechanism. In *she1Δ* mutants, dynactin is enriched at plus ends throughout the cell cycle, increasing the local ratio of dynactin:dynein [30, 33]. Conversely, dynactin is depleted from plus ends in cells overexpressing She1 [31]. This effect is specific to dynactin recruitment. She1 does not prevent dynein from accumulating at plus ends, even under overexpression conditions [30, 31, 33]. Moreover, She1 does not alter formation or stability of the dynactin complex [31]. Instead, She1 may regulate the association of dynactin with dynein at the microtubule plus end.

She1 directly regulates dynein motility

A recent paper by Markus and colleagues adds a new dimension to She1 function. She1 localizes to spindle and astral microtubules in cells, and binds directly to microtubules in vitro [30, 31, 34]. Binding requires the negatively-charged carboxy-terminal tails of α - and β -tubulin [32]. This raises the possibility that She1 may influence dynein's interaction with microtubules. To test this, Markus and colleagues evaluated the effect of She1 on dynein motility using in vitro assays measuring the movement of single molecules of dynein heavy chain. In this assay, adding an increasing concentration of She1 slows dynein movement until the motors are nearly still on the microtubule [32]. Stalled dynein motors exhibit prolonged dwelling on the microtubule. Experiments with a minimal motor domain of dynein heavy chain, which lacks the tail domain, yield similar results. These effects were not

^{*}The *SHE1* gene described here is named for Sensitivity to High Expression [44], systematic name YBL031W. This is not to be confused with the type V myosin, *MYO4*/YAL029C, which was identified as a Suppressor of the HO Endonuclease, and thus named *SHE1* [47].

seen for kinesin motors exposed to high levels of She1. This indicates that She1 directly and specifically alters dynein motility.

How might She1 inhibit dynein motility? Although the details of the She1 mechanism remain unresolved, let us consider a few speculative models. One model is that She1 could block the advance of dynein by occupying or obstructing its binding site on the microtubule (Fig. 2A). Dynein would then dwell at one position because of decreased availability of the forward position. An interesting comparison here is the Tau protein, which can block the advance of dynein in this manner [13]. Several lines of evidence argue against such a model for She1. First, She1 does not prevent dynein from binding to microtubules. In vitro, high levels of She1 do not prevent dynein from loading onto microtubules; in vivo She1 overexpression does not impair dynein localization to microtubules [31, 32]. Second, dynein and kinesin are thought to share overlapping binding sites on the microtubule, yet She1 does not inhibit kinesin [32, 35]. Third, She1-5D, which mimicks phosphorylation by Ipl1 kinase, exhibits weaker microtubule-binding and faster diffusion along the microtubule [32]. If She1 occludes dynein's binding site, then She1-5D might permit greater access. To the contrary, She1-5D is a potent dynein inhibitor [32]. This suggests that She1 does not compete with dynein for a common binding site.

A more likely model is that She1 binds directly to dynein (Fig. 2B). Consistent with this, Markus and colleagues use dual-label single molecule experiments to demonstrate that when dynein encounters She1 on the microtubule, they dwell together, each inhibiting the movement of the other [32]. These encounters hint at a She1-dynein complex; however, a direct interaction has not been demonstrated. Nevertheless, it is tempting to speculate that She1 selectively binds a conformation- or perhaps nucleotide-state of the dynein motor, stabilizing its interaction with the microtubule.

This model is reminiscent of how LIS1 regulates dynein. LIS1 binds to the dynein motor domain and decreases its velocity in vitro [36, 37]. However, in contrast to She1, LIS1 is necessary for dynein functions in a variety of cellular contexts across species, suggesting LIS1 is a positive regulator. If LIS1 and She1 have similar effects on dynein in vitro, why do they have opposite effects on dynein in cells? The answer may lie in how She1 and LIS1 bind to the motor domain. Although LIS1 slows dynein's movement along the microtubule, it does not dramatically affect ATPase activity; therefore, LIS1 does not lock dynein in a specific nucleotide state [36]. Instead, LIS1 alters the cascade of nucleotide-dependent conformational changes within the motor domain, preventing dynein from dissociating from its microtubule track upon binding ATP [36]. This slows the rate of dynein stepping, but allows dynein to generate greater force, presumably by decreasing the likelihood that dynein will detach from the microtubule [36, 37]. Whether She1 alters dynein's ATPase activity is an important question, and would point to a mechanism for inhibiting motility.

She1 in the cell – separate activities or a unified mechanism?

Markus and colleagues then investigate how She1 regulates dynein activity in cells. By measuring dynein-dependent spindle movement in S-phase arrested cells, they find that *she1Δ* null mutants exhibit more frequent movement; however, compared to cells expressing wild-type *SHE1*, these movements were biased toward the mother compartment and rarely drew the spindle into the bud [32]. A caveat of these experiments is that they were conducted in a *kar9Δ* mutant background, which elicits hyperactive dynein through an independent mechanism that is poorly defined [24, 29, 30]. Thus, wild-type controls in these experiments possess hyperactive dynein. Nevertheless, the results suggest the role of She1 may be to direct dynein activity toward the bud, in order to promote bud-ward spindle movement.

If She1 promotes polarized force generation, how do cells regulate She1? In one model, the abundance of She1 on astral microtubules could control its activity. Consistent with this, the localization of She1 to astral microtubules is decreased during mitosis, when dynein activity is greatest [22, 27, 30]. This change in She1 localization might be linked to its phosphorylation status. She1 is phosphorylated by the Ipl1 kinase [32, 38]. Ablating five predicted Ipl1-consensus sites in She1 (She1-5A) elicits greater dynein activity in the spindle movement assay [32]. Conversely, phospho-mimetic mutations (She1-5D) do not exhibit a strong phenotype in cells, but weaken She1's affinity for microtubules and promote faster diffusion along the microtubule lattice in vitro [32]. This recalls the behavior of Ndc80 and the Dam1/DASH complex, which also interact with negatively-charged tubulin tails and exhibit faster lattice diffusion after phosphorylation within the microtubule-binding domain [39, 40]. At this point, the microtubule-binding domain of She1 is not experimentally defined or predicted by sequence homology, and the five predicted Ipl1 sites are peppered throughout She1. Furthermore, we do not know how phosphorylation affects She1 localization in cells. These questions will require further investigation, including an examination of how cell polarity signaling may affect She1.

The effect of She1 on the dynein motor in vitro must also be reconciled with other roles of She1 in cells. The most pressing question is whether motor regulation is related to dynactin recruitment. Dynactin associates with the dynein complex by binding to the dynein intermediate chain; interaction with dynein's motor domain has not been identified [41–43]. That She1 inhibits two activities of dynein – motility and dynactin recruitment – therefore suggests that She1 may act at multiple sites – the motor domain and the complex of the tail domain and intermediate chain. Previous work by Markus and colleagues show that the activities of these two sites are linked. Dynein can be ectopically activated in cells either by expressing separate fragments of the motor and tail domains or by inserting a steric linker between the two domains [44, 45]. This leads to the model that dynein is regulated by alternating between a 'locked' state, where the motor and tail domains are simultaneously inhibited, and an 'unlocked' state where the motor domain is motile and the tail domain supports recruitment of dynactin via the intermediate chain. If She1-regulation of the motor domain contributes to maintenance of the 'locked' state, this might explain the inhibition of both motor activity and dynactin recruitment (Fig. 2C).

Adding further complexity to this story, recent work suggests an additional role for She1 in promoting posttranslational modification of LIS1. In yeast, LIS1 is modified by SUMOylation and ubiquitylation; these modifications are diminished in *she1Δ* mutants [46]. Similar diminution of LIS1 modification was observed in null mutants in *KAR9* and *BIM1*, the yeast EB1 homologue [46]. Although the consequences of these modifications for LIS1 activity are not yet known, it is interesting to note that all three mutants – *she1Δ*, *kar9Δ*, and *bim1Δ* – exhibit hyperactive dynein [29, 30]. This hints at some crosstalk between She1 and LIS1 that may control dynein activity.

Finally, several results indicate that She1 has roles in the cell that are dynein-independent. *SHE1* was originally identified in a screen for genes that are Sensitive to High Expression [47]. Cells overexpressing *SHE1* exhibit poor growth and accumulate with a duplicated genome and medium-to-large buds, indicative of cell cycle delay in G2 or mitosis [48]. Mutants that disrupt dynein do not commonly exhibit this cell cycle delay; therefore it is likely that the *SHE1* overexpression phenotype is independent of dynein. Indeed, *SHE1* overexpression remains toxic in *dyn1Δ* null mutants [32]. She1 interacts with several kinetochore proteins, including components of the Dam1/DASH complex (Dam1, Duo1, Spc34) and the chromosome passenger complex protein Sli15 [34, 49]. In addition to dynein phenotypes, *she1Δ* mutants show delayed spindle disassembly [38]. Thus, She1 appears to promote the disassembly of spindle MTs. This requires the AAA+ ATPase Mcm2 and

phosphorylation of She1 by Ipl1 [38]. Dynein is excluded from the yeast spindle, perhaps because it is not actively transported through nuclear pores; therefore the role of She1 in regulating spindle stability must be dynein independent. Whether this involves a mechanism similar to dynein regulation is unknown.

Concluding remarks

How cells direct a biochemical activity, such as dynein motility, to produce spatially and temporally precise outcomes is an important unresolved question in cell biology. The studies discussed here highlight one aspect of a complex pathway for directing dynein activity to accurately position the spindle and nucleus in yeast cells. An important question is: How pervasive is the She1 mechanism? She1 homologues are conserved in other hemiascomycete yeasts; but orthologues in distant species have yet to be identified. In contrast, obligate regulators of dynein - including chains of the dynein motor, dynactin, and LIS1 - are well-conserved across species. Perhaps the mechanisms for fine-tuning dynein have diversified through evolution in order to suit different cellular contexts; in which case, regulators like She1 may only be found in yeast. Alternatively, it may simply be difficult to identify these regulators experimentally. For example, loss of She1 in cells elicits a subtle defect in dynein activity that is revealed by isolating dynein-dependent spindle movement. The increasing sophistication and sensitivity of *in vivo* models and their *in vitro* counterparts will no doubt continue to unveil the complexity of dynein regulation, and keep us busy in the meantime.

Acknowledgments

I am grateful to the members of the Moore, McMurray, and Pearson labs (UC-SOM) for helpful discussions in the preparation of this manuscript. This work was supported by a grant from NIH (R00GM092968).

Abbreviations

AAA+ ATPase	ATPase Associated with diverse cellular Activities
EM	Electron Microscopy
MTBD	Microtubule Binding Domain
SPB	Spindle Pole Body
SUMO	Small Ubiquitin-related Modifier

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Figure 1. Dynein delivers a genome and nucleus to the yeast bud

1) Dynein is targeted to the plus ends of astral microtubules. A complex of kinesin/Kip2 and CLIP170/Bik1 transport dynein toward the plus end [50, 50]. Dynein is retained at plus ends by LIS1/Pac1 and NudEL/Ndl1 [51, 52]. At this point, dynein is held in an inactive, or locked, state [44, 45]. 2) At the plus end, unlocked Dynein recruits dynactin [26]. 3) Dynein-dynactin surfs the plus end of the dynamic as it grows and shrinks, until it docks with its cortical receptor, Num1 [51, 53]. 4) Cortically anchored dynein walks toward the minus-end of the astral microtubule, generating tension that pulls the spindle pole toward the anchored motor [22, 51].

Figure 2. Models for She1 inhibition of dynein

A. She1 occludes dynein's binding site on the microtubule, preventing the advance of the motor. B. She1 binds directly to the dynein motor domain and stabilizes its interaction with the microtubule. C. She1 binds to dynein, stabilizing a conformation that inhibits the motility of the motor domain and the recruitment of dynactin by the intermediate chain. These models are not mutually exclusive.