Activation of the motor protein upon attachment: Anchors weigh in on cytoplasmic dynein regulation

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Cytoplasmic dynein is the major minus-end-directed motor protein in eukaryotes, and has functions ranging from organelle and vesicle transport to spindle positioning and orientation. The mode of regulation of dynein in the cell remains elusive, but a tantalising possibility is that dynein is maintained in an inhibited, non-motile state until bound to cargo. In vivo, stable attachment of dynein to the cell membrane via anchor proteins enables dynein to produce force by pulling on microtubules and serves to organise the nuclear material. Anchor proteins of dynein assume diverse structures and functions and differ in their interaction with the membrane. In yeast, the anchor protein has come to the fore as one of the key mediators of dynein activity. In other systems, much is yet to be discovered about the anchors, but future work in this area will prove invaluable in understanding dynein regulation in the cell.

Keywords:
cytoplasmic dynein; dynein anchor; motor regulation; ternary complex

Introduction

Precise positioning of the nucleus during cell division is important for proper segregation of chromosomes and determination of cell fate. A common theme across different organisms is the employment of the association between cortical cytoplasmic dynein motors and the astral microtubule of the spindle for positioning events. Microtubule-associated cytoplasmic dynein, the ubiquitous minus-end-directed motor protein, is responsible for cargo, organelle and RNA transport during interphase and spindle positioning and orientation during mitosis and meiosis [1–3]. The former is achieved when cargo-bound dynein translocates on microtubule tracks in the cell, moving towards the minus-ends of the microtubule. The latter occurs when cortically anchored cytoplasmic dynein exerts forces on microtubule plus-ends that extend towards the cell periphery. This results in pulling of the centrosome, and hence, the attached nuclear material [4].

Cytoplasmic dynein is a large protein complex that consists of a homodimer of dynein heavy chains and several accessory proteins (Fig. 1). Dynein heavy chain (HC) is a ~500 kDa protein that has a C-terminal ring of 6 AAA+ domains and the microtubule binding domain and an N-terminal cargo-binding and dimerisation domain [5, 6]. Of the 6 AAA+ domains, AAA1 is the primary site of ATP binding and hydrolysis which translates the energy from ATP to mechanical energy [7]. The linker domain of dynein extending between AAA1 and the tail domain, changes its position across the AAA ring upon ATP hydrolysis and is thought to be the mechanical element based on electron microscopy data [5, 8–12]. The N-terminus of HC is the site for dimerisation and binding of the accessory proteins-intermediate chain (IC) and light intermediate chain (LIC). Other accessory proteins, the light chains (LC) bind to the IC of the dynein complex. Dimerisation of the HC is essential for the property of processivity of dynein, which is the ability of the motor to take several steps on the microtubule before detachment [13]. In addition to cargo binding, the N-terminal region of dynein has been implicated in attachment to the anchor protein/protein complexes either directly or indirectly.

Dynein regulators dynactin and Lis1 modulate the activity of dynein by increasing the processivity and the load-bearing capacity of dynein, respectively. Dynactin is a 1.2 MDa protein complex that interacts with the HC N-terminal tail and IC [6, 14–16] (Fig. 1), and causes an increase in dynein processivity [17]. The dimeric protein Lis1 binds to the motor head of

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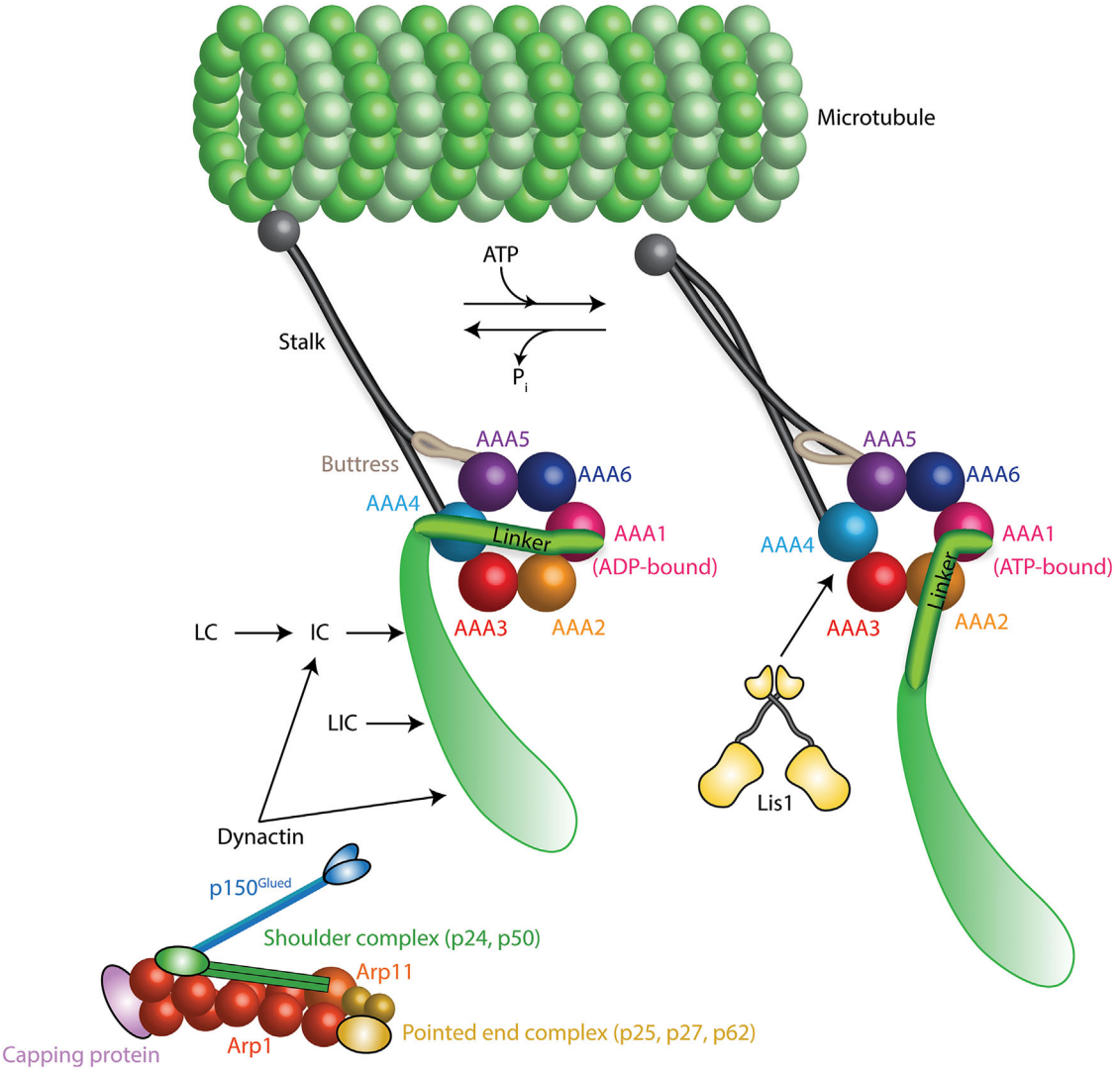

Figure 1. Schematic depicting the dynein heavy chain (HC) and its regulators dynactin and Lis1. Binding andhydrolysis of ATP on the HC AAA1 on the C-terminus leads to unbinding of the microtubule binding domainfrom the microtubule. The N-terminal tail region of HC is site for binding of the IC, LIC and dynactin. The LCin turn binds to the IC. Lis1 binds to a region between AAA3 and AAA4 of the HC.

dynein between AAA3 and AAA4 [18] (Fig. 1) and is thought to act as a 'clutch' by uncoupling dynein's ATP hydrolysis from microtubule binding, thereby stabilising the interaction between dynein and the underlying microtubule [18, 19] and increasing dynein's ability to transport large cargo over long distances.

A sea of information regarding dynein's force-production, stepping and processivity has been obtained from studies on purified yeast dynein, which is a robust minus-end-directed motor protein [13]. Mammalian dynein's properties, however, have been described variously by different groups [20–24]. Recent work with recombinant human and rat dynein in vitro has identified that mammalian dynein is primarily non-processive in motility assays, even in the presence of ATP [25–29]. Whereas groups of dynein motors stuck to glass slides exhibited robust movement along microtubules in gliding assays, single dynein motors did not show directed movement in a conventional motility assay [25]. Dynein adaptors BicD2, Spindly, Rab11-FIP3 and Hook3, that link dynein to dynactin were found to be able to switch dynein's movement from diffusive to directed, with accumulation of dynein at the minus-ends of the microtubule, indicating tenacious binding of dynein to the microtubule [27, 28, 30, 31].

Even though yeast dynein was capable of minus-end-directed motility in vitro, inside the cell, the association of yeast dynein with its anchor protein was demonstrated to activate dynein [32, 33]. However, the sole function of dynein in these systems is in the anaphase nuclear migration and meiotic oscillation, respectively. In the budding yeast system, the association of dynactin with dynein and the simultaneous dissociation of Lis1 ortholog Pac1 were found to be required to activate dynein motility [32]. In fission yeast, while dynactin was found to be essential for dynein's association with the anchor protein, no orthologs of mammalian Lis1 have been identified yet.

Taken together, dynein's activity is regulated in vivo by a combination of regulators of dynein, the adaptors of cargo proteins of dynein, and the cargoes themselves (e.g. anchor proteins). The microtubule plus-end-directed motor protein, kinesin, has been demonstrated to be in an auto-inhibited conformation until bound by cargo protein [34]. An interesting possibility is that dynein inside the cell is also in a default 'inactive' state until bound by cargo proteins or adaptor proteins [6, 16, 29, 35]. In this review, I will focus on six systems including fungi and metazoans that involve the activity of cortical dynein and its anchor protein for nuclear arrangement. The modes of regulation of dynein by these anchor proteins and the possible mechanisms by which the regulation occurs are also discussed.

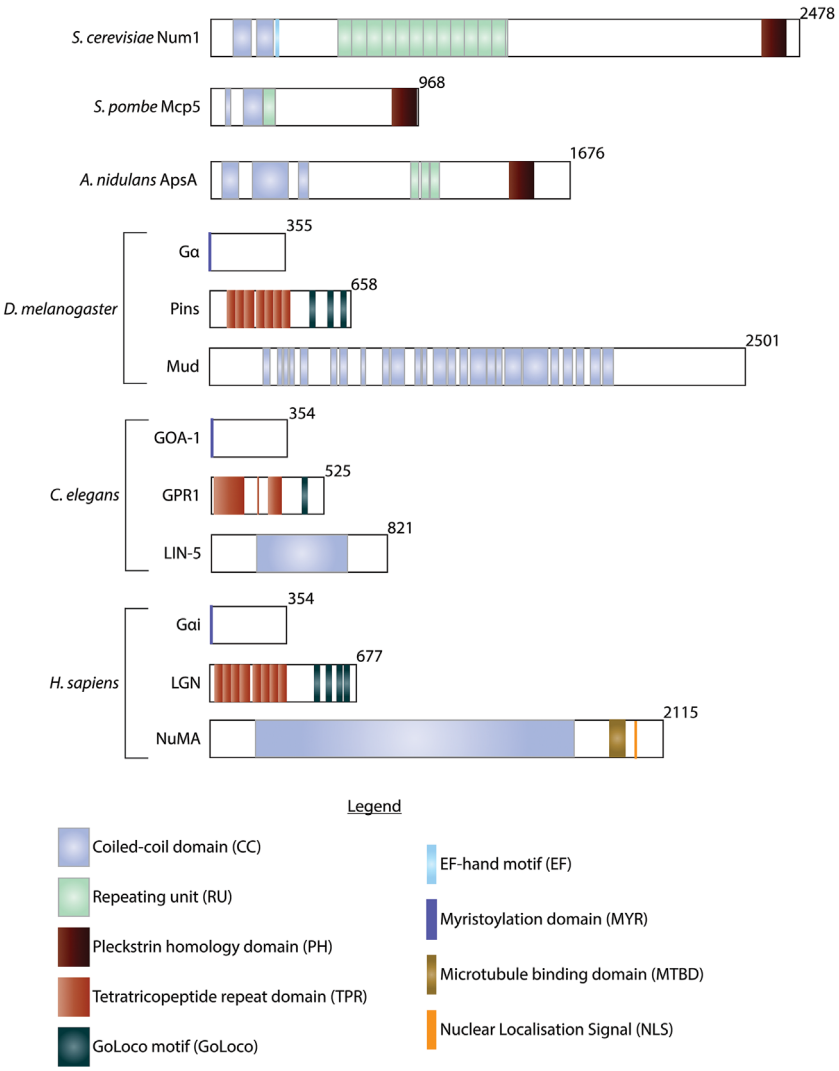

Figure 2. Sequence maps of the anchor proteins and ternary complexes that are required for cortical localisation of dynein. Numbers to the right of each sequence map indicate the amino acid length of that particular protein.

Anchor proteins in fungi are coiled-coil proteins with a membrane-targeting pleckstrin homology domain

Cytoplasmic dynein in *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Aspergillus nidulans* employs anchor proteins Num1, Mcp5 and ApsA, respectively, to mediate their attachment to the membrane. These anchor proteins contain a conserved pleckstrin homology (PH) domain at the C-terminus, which is required for membrane localisation and an N-terminal coiled-coil (CC) domain that is required for association with dynein [36–39] (Fig. 2). Num1 in addition has a central region of 13 repeating units of 64 amino acids and a Ca^{2+}-binding EF-hand at the N-terminus [36]. Mcp5 shares 27% homology with Num1 and a conserved repeating unit (RU) [37, 38].

Budding yeast Num1 is required for anaphase spindle orientation

Budding yeast Num1 was identified as a 313 kDa protein that anchors dynein at the membrane [36, 40]. Num1 appears as immobile punctae on the membrane of mitotic cells, and is essential for capturing and sliding of astral microtubules of the spindle [36]. In *S. cerevisiae*, the assembly of the mitotic spindle in a dividing cell occurs in the mother cell, from where it traverses the bud neck for proper cell division [41]. This migration of the spindle occurs in two steps employing distinct sets of proteins. The first is a pre-anaphase process dependent on the microtubule plus-end tracking Bim1 and microtubule-binding Kar9 that aid the movement of the spindle from the mother cell to a site adjacent to the bud neck [42, 43]. The second migration is required for the positioning of the spindle between the mother and the bud cell during anaphase, which is a dynein(Dyn1)/Num1-dependent process [44–48] (Fig. 3A). Mutants lacking Num1 are viable, but exhibit impaired nuclear migration into the daughter cell during mitosis [36]. The current model of the spindle positioning during anaphase relies on the 'off-loading' of the dynein-dynactin complex to Num1 via targeting to the plus-ends of astral microtubules in a Bik1 (CLIP-170)/Pac1(Lis1)-dependent fashion [46, 48, 49]. Unlike CLIP-170 which tracks the growing plus-ends of microtubules, Bik1 tracks both growing and shrinking plus-ends because of the involvement of the kinesin Kip2 in the plus-end localisation of Bik1 [48]. Upon being off-loaded to the cortex, dynein is activated and pulls on the astral MT to position the spindle [46, 50, 51].

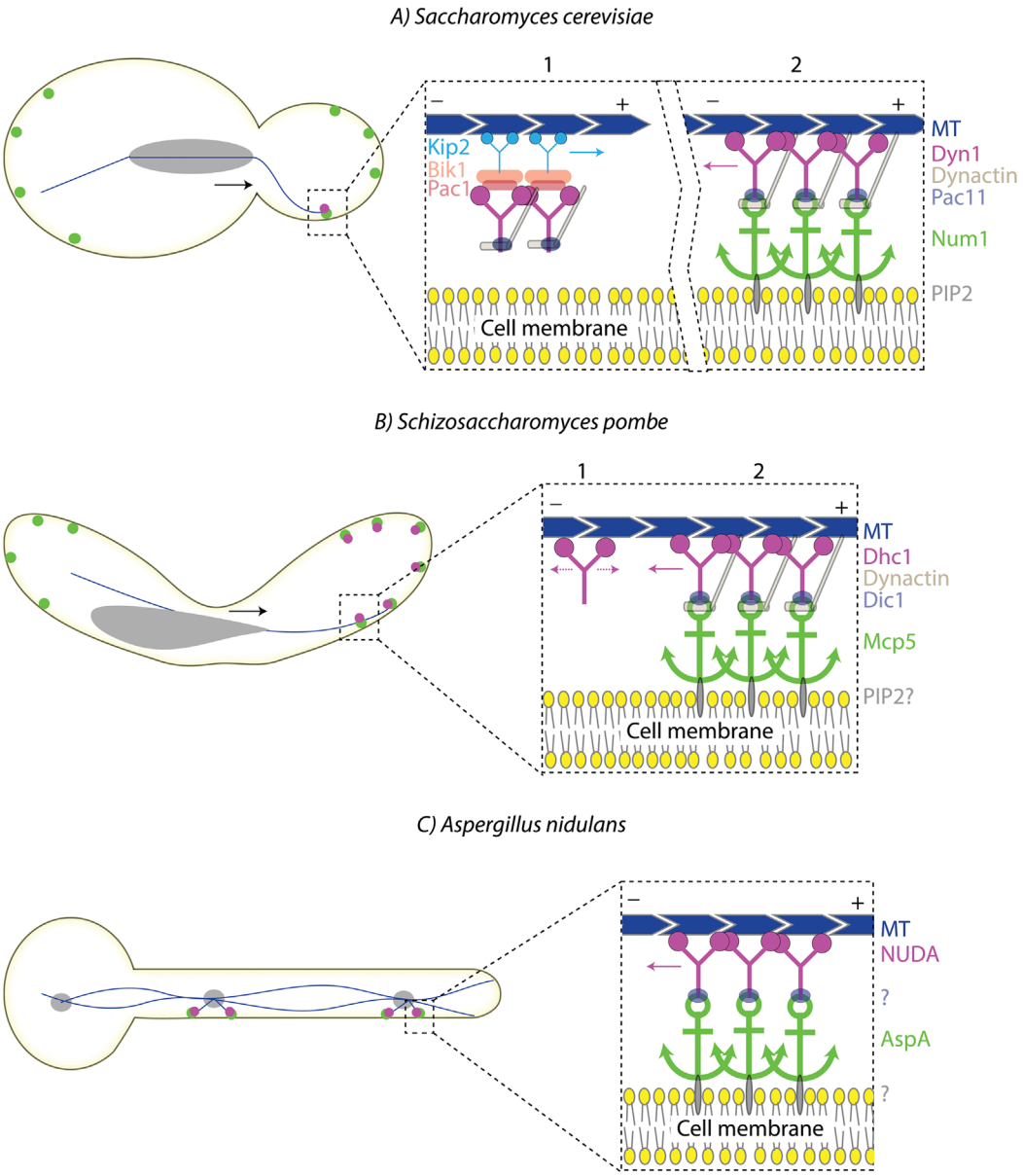

Figure 3. Overview of the fungal anchor proteins and their function in dynein-mediated nuclear organisation and positioning events. **A:** During anaphase in budding yeast, dynein-dynactin (magenta-beige) is first targeted to the plus end of the microtubule (blue) in a Kip2 (light blue) and Bik1/Pac1 (light/dark orange)-dependent fashion, from where it is offloaded to cortical Num1 (green) bound to PIP_2 (grey) in step 1. In step 2, the dissociation of Pac1 from the dynein complex and concomitant attachment of dynein-dynactin to Num1 activates dynein for pulling on the plus end of astral microtubule and results in the movement of the anaphase spindle into the bud. **B:** Mcp5 (green) is required for oscillatory nuclear movements during prophase of meiosis I in fission yeast, when dynein-dynactin binds to cortical Mcp5 likely via Dic1 subunit (blue). The proposed binding of Mcp5 to the membrane is via PIP_2 (grey). **C:** ApsA (green) is essential for nuclear distribution *A. nidulans* hyphae, although the precise subunit of dynein to which ApsA binds and the membrane interacting-partner of ApsA is unknown. The cell membrane is depicted as a bilayer (yellow), direction of movement of the nucleus (grey) is indicated by the black arrow and direction of dynein walking is indicated by the magenta arrow in the insets.

The targeting of Num1 to the membrane is mediated by PIP_2

The PH domain of Num1 has high selective affinity for the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP_2) in vitro [52]. While the PH domain's affinity for PIP_2 was necessary and sufficient for membrane localisation of Num1, the distinct punctate localisation of Num1 bound to the membrane was absent in strains exhibiting solely the PH domain from Num1 [53]. The PH domain-mediated targeting of Num1 was expendable in a mutant strain where the PH domain was replaced with the membrane-binding CAAX domain from Ras protein [53]. In such a strain, both the punctate phenotype of Num1 localisation and the functionality of the hybrid Num1 in positioning of the mitotic spindle were rescued. In later experiments, it was observed that deletion of the first CC (CC1, Fig. 2) at the N-terminus of Num1, leads to the disperse localisation of Num1-CC1Δ-GFP at the

membrane [54]. This CC1 domain was found to harbour a Bin/Amphiphysin/Ras-like (BAR) domain, which was necessary and sufficient for the clustering or formation of patches of Num1 at the membrane [54].

The anchor protein of dynein in *S. pombe* is required for meiotic nuclear oscillations

The homolog of Num1 in fission yeast was identified as the 111 kDa *m*eiosis-specific *c*oiled-coil *p*rotein 5 (Mcp5) in a large screen of proteins required for meiosis [55]. The expression of Mcp5 was found to be restricted to meiotic prophase [37, 38]. Meiosis in fission yeast leads to the fusion of haploid cells of opposite mating types, followed by karyogamy or fusion of their nuclei to form a zygote. The fused nucleus is then led in an oscillatory back-and-forth movement between the two poles of the zygote, powered by dynein pulling on microtubules emanating from the SPB [56, 57] (Fig. 3B). Fluorescence microscopy of dual-labelled Mcp5 and dynein HC (Dhc1) has established this movement to be dependent on Mcp5-mediated localisation of dynein to the cortex [37, 38]. Elegant microtubule laser ablation experiments confirmed that the cortical dynein motors apply a pulling force on the microtubule along its length [58]. The dynactin subunit Ssm4 ($p150^{Glued}$ in vertebrates) was observed to be required for anchoring of dynein at the cortex [59]. More recently, the dynein-anchoring function of Mcp5 was reinforced by the observation of assemblies of dynactin subunits Mug5, Mug1 and Arp1 (p24, p50/dynamitin and Arp1 in vertebrates) with Mcp5 at the cortex [60]. While binding of Mcp5 to dynein was independent of dynactin, the cortical anchoring of dynein relied on the presence of dynactin. In cells lacking Mcp5, these oscillations cease, leading to improper chromosome pairing and recombination and, subsequently, sporulation defects [37].

Mcp5 mutants lacking the CC domain or RU were found to be able to localise to the cell membrane, but exhibited aberrant nuclear oscillations similar to the Mcp5 deletion mutant [37]. Mutants of the PH domain were unable to localise to the cortex, and thereby, also failed to drive oscillatory movement of the nucleus [37, 38]. All three mutants were defective in spore formation, indicating that the CC, RU and PH domains are essential to the function of the anchor protein and its involvement in meiosis [37, 38]. The attachment of Mcp5 to the membrane is speculated to be via the PH domain to PIP_2 [37], as in budding yeast.

The anchor protein ApsA is required for nuclear distribution in *A. nidulans*

In the filamentous fungi *A. nidulans*, nuclear migration and distribution occur in a microtubule-mediated dynein-dynactin pathway [61] (Fig. 3C). The homolog of Num1/Mcp5 in this model system was discovered to be the 183 kDa protein named ApsA [39] (Fig. 2). ApsA is also found to localise to the cortical membrane, likely via its PH domain, although the precise targeting mechanism is unknown. In mutants of dynein-dynactin, nuclear distribution and conidiophore (spore-bearing structure of filamentous fungi) formation are severely impaired, whereas in ApsA mutants, the effect is much milder [39, 62, 63]. This observation hints that dynein (NUDA) might be required as a tether for the plus-ends of microtubule at the cortex, rather than an actual force-producing entity [64]. Dynein is found along with Lis1/Pac1 homolog NUDF in comet-like structures at the dynamic ends of microtubules in hyphal tips of *A. nidulans* [61, 65]. Mutations within the AAA1 and AAA3 of dynein C-terminal domain that affected endosome movement were surprisingly normal in their nuclear distribution [64]. This gave rise to the hypothesis that dynein's activity, which is required over longer periods of time for nuclear distribution, need not be robust, and that a low level of activity would be sufficient, whereas the same mutation would be detrimental for endosome and cargo transport which happen over shorter time durations [64].

Dynein motility in fungi is activated upon binding to the anchor at the cortex

Recent studies in yeast have reported a novel regulation of dynein activity by their attachment to the anchor protein [32, 33]. While dynein was found to be inactivated when bound to microtubule alone, upon attachment to both microtubule and anchor protein, dynein started to move in a minus-end-directed fashion.

Attachment to Num1 at the cortex alleviates Lis1 inhibition of dynein in budding yeast

Since dynein localises as spots on the plus-ends of budding yeast astral microtubule owing to targeting via Bik1/Pac1, it was hypothesised that dynein activity was somehow suppressed until it was off-loaded to the cortex [47]. When dynein's C-terminal motor domain and N-terminal tail domain were expressed independently as GFP-tagged constructs, the tail-GFP domain localised directly to the membrane-bound Num1, whereas the motor domain was targeted to the plus-ends, but never off-loaded to the cortex [50]. This observation suggested that there was a masking of the tail domain by dynein head domain or its associated proteins, which prevented the offloading of dynein to the cortex until the unmasking event at the plus-ends of the microtubule. More recently, it was discovered that this unmasking event correlates to the observation that once plus-end localisation of dynein-dynactin is achieved, offloading of the complex to the cortex and binding to Num1 dissociates the Lis1 ortholog Pac1 from dynein [32]. The dissociation of Lis1 was critical for the activity of dynein bound to Num1. Interestingly, in cells overexpressing the CC domain of Num1 (Num1-CC), the Lis1-mediated dynein inhibition was overcome. Lis1 binds to a region between AAA3 and AAA4 of dynein HC's motor domain [18]. Therefore, the effect of Num1-CC overexpression on dynein-Lis1 binding is proposed to be an outcome of allosteric inhibition since Num1 and Lis1 do not bind to the same region on dynein HC [32]. Further, dynactin's association

was shown to be critical for dynein activity, because in deletion mutants of dynactin, there was increased plus-end localisation of dynein at the plus-end of astral MT [47, 66]. The specific interaction between budding yeast dynein and Num1 is unknown, but pull-down experiments with the CC of Num1 have identified the IC to be the likely candidate [54].

Dynein switches from inactive to active state upon binding Mcp5 in fission yeast

Direct observation of single dynein molecules [67, 68] in fission yeast led to the discovery of a two-step process in the targeting of dynein to the anchor, with binding to the microtubule acting as an intermediary [33, 58]. Interestingly, while dynein exhibited three-dimensional diffusion in the cytoplasm and slow one-dimensional diffusion on the microtubule, it switched to directed movement upon binding Mcp5 [33]. This model was reinforced by demonstrating in a PH domain deletion mutant – where cortical localisation of Mcp5 was abolished – that dynein bound to Mcp5-CC on the microtubule was capable of minus-end-directed movement towards the SPB. This finding lead to the conclusion that binding of dynein to the anchor Mcp5 relieved the inhibition of dynein's motor activity and enabled it to move in a minus-end-directed fashion. In recent work, it was suggested that this activation upon anchoring also involves assembly of dynactin subunits Mug5 (p24), Jnm1 (p50) and Arp1 based on accumulation of dynein at the plus-end of microtubules in mutants of the dynactin subunits [60], similar to what was observed in budding yeast [46]. More experiments with mutants of the dynein motor will be required to identify the minimal dynein complex required for processive directed movement. In budding yeast, the activation of dynein was demonstrated to be the result of removal of Lis1-mediated inhibition upon anchoring [32, 47].

In *S. pombe*, the Lis1 homolog is yet to be discovered, and it remains unclear whether a similar mechanism operates in dynein activation. The dynein IC (Dic1) co-localised with the HC in fluorescence microscopy [69], and immunoprecipitated with Mcp5 independent of dynactin [60]. This likely indicates an interaction between the dynein complex and Mcp5 mediated by IC, similar to that found in the budding yeast system [54]. Interestingly, the anchoring of dynein to Mcp5 was not observed in strains expressing dynein HC mutated in its first AAA+ domain, suggesting that active dynein is required for its anchoring [60]. This unbinding of the mutated dynein was proposed to occur due to high load forces exerted upon anchored mutated dynein by the shrinking of microtubules [60].

Could cortical dynein be required solely as a tether for microtubules in *A. nidulans*?

While there is still no evidence for direct interaction between dynein-dynactin and ApsA in *A. nidulans*, two theories exist on how the process of nuclear migration and distribution might be mediated by an interaction between dynein-dynactin and ApsA. In the first instance, dynein bound to ApsA could act as a tether for microtubules [70, 71], whereupon microtubule depolymerisation could drive the movement of nuclei. In the second case, as in budding and fission yeast, ApsA-bound dynein-dynactin could actively drive the movement of the nucleus by pulling on microtubules. More experiments are required for elucidation of the precise mechanism of nuclear distribution involving dynein, dynactin, Lis1, ApsA and their interaction.

A ternary complex operates in flies, worms and mammals to keep dynein at the cortex

While fungal systems rely on a single anchor protein to localise dynein at the cell cortex, metazoans employ a complex of three proteins – 'the ternary complex' – to perform this function. The ternary complexes are evolutionarily conserved and bear strikingly similar functions across the systems in which they operate. In the ternary complexes, the functions of dynein interaction and membrane interaction are split amongst the three protein modules that make up the complex [72]. Cortical anchoring of dynein via the ternary complex facilitates asymmetric positioning of the spindle in *Drosophila* and *Caenorhabditis elegans*, and centring of the *Homo sapiens* mitotic spindle (reviewed in Refs. [72–74]).

The ~2500-amino acid (aa) long *Drosophila* Mud is the functional ortholog of ~800-aa long *C. elegans* LIN-5 and ~2100-aa long mammalian NuMA [75, 76] (Fig. 2). All three proteins contain a central large CC domain [77]. NuMA, in addition, has a C-terminal microtubule-binding domain (MTBD) and nuclear localisation signal (NLS) [78–80]. Mud/LIN-5/NuMA interact with the TPR-GoLoco containing proteins Pins/GPR-1/2/LGN [81, 82]. Heteromeric G proteins Gα/Gα/Gαi in *Drosophila melanogaster*/*C. elegans*/*H. sapiens*, respectively, in turn bind to Pins/GPR-1/2/LGN and target them to the membrane through a myristoylation domain at the N-terminus. Partner of Inscuteable (Pins), binds to the protein Inscuteable (Insc) and interacts with Mud via its TPR region and with GDP-Gαi through the GoLoco region [83]. In mammalian cells, the binding of GDP-bound Gαi to LGN relieves the auto-inhibition between the GoLoco and TPR domains thereby making it available for association with NuMA [84]. NuMA's binding to its partner LGN is via a site that overlaps with the MTBD. Thus, NuMA's dual role in MT bundling and LGN-binding might be regulated due to competition between the MT and LGN for this shared region [85, 86].

The ternary complex in *Drosophila* is required during asymmetric division of the neuroblast

In *Drosophila*, asymmetric division of the neuroblast is required for proper neuronal development. Each neuroblast undergoes division to give rise to another neuroblast and a ganglion mother cell which in turn gives rise to neurons and glia. For the asymmetric apical-basal orientation of the mitotic spindle, the ternary complex Mud/Pins/Gα is

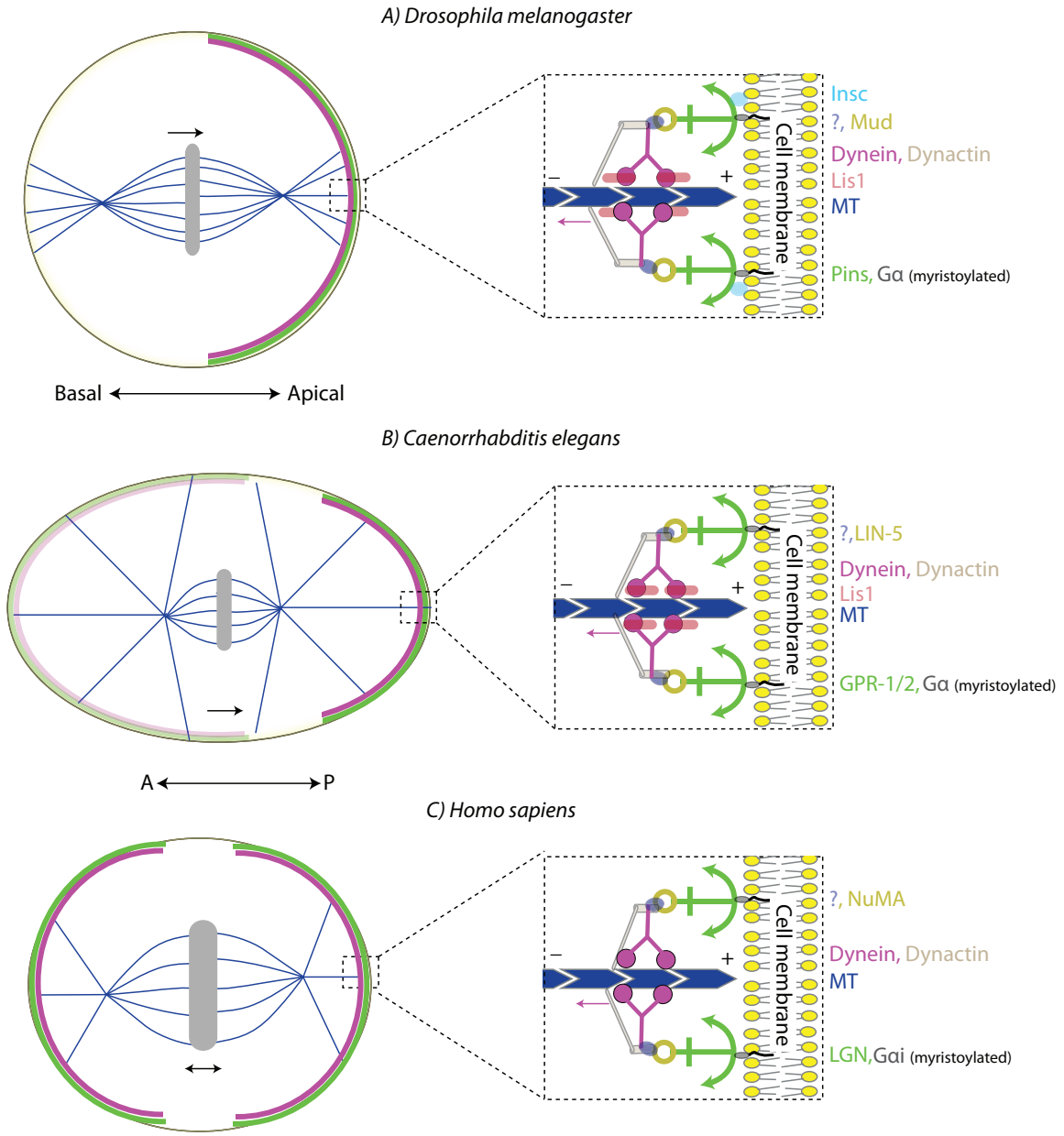

Figure 4. Overview of the ternary complexes and their function in dynein-mediated nuclear positioning and orientation events. **A:** In *Drosophila* neuroblasts, the apical positioning of the spindle along the apical-basal axis (A<->B) is ensured by the cortical dynein-dynactin (magenta-beige) and Mud/Pins/Gα ternary complex (green, light green, grey). The scaffold protein Insc (light blue) ensures the interaction between the membrane and Mud. **B:** Dynein-dynactin (magenta-beige) and the ternary complex LIN-5/GPR-1/2/Gα (green, light green, grey) are essential for the asymmetric localisation of the mitotic spindle in *C. elegans* one-celled embryo towards the posterior along the anterior-posterior (A<->P) axis. **C:** In human cells, cortical dynein-dynactin (magenta-beige) bound to NuMA/LGN/Gαi (green, light green, grey) is essential for the central localisation of the metaphase spindle. The cell membrane is depicted as a bilayer (yellow), direction of movement of the nucleus (grey) is indicated by the black arrow and direction of dynein walking is indicated by the magenta arrow in the insets.

required [76, 83, 87, 88] (Fig. 4A). Mud/Pins/Gα localises to the apical cortex in the dividing neuroblast [74] mediated by the scaffold protein Canoe (Cno) that interacts with small GTPases [89]. Dynein HC (Dhc64c) is essential for the apical-basal spindle orientation, as a misorientation phenotype was observed in mutants of HC [90]. While primary components that are essential for the asymmetric positioning of the spindle have been identified, the spatio-temporal regulation of the ternary complex proteins is yet to be explored in *Drosophila*.

Asymmetric division of the one-celled embryo of *C. elegans* requires the ternary complex

In the *C. elegans* one-celled embryo, the assembly of the spindle is initiated in the cell centre, but subsequently

traverses to the posterior of the cell during anaphase as a result of differential localisation of polarity markers along the anterior-posterior axis. This results in asymmetric division of the embryo into the AB and P1 blastomeres and is critical for the proper development of the embryo [91]. In addition to the polarity markers that provide the cue for the asymmetric division [92], the association of dynein with the ternary complex of LIN-5/GPR-1/2/Gα is essential for this process [93–95] (Fig. 4B).

The association between Gα and the GoLoco domains of GPR-1/2 is possible only when Gα is in the GDP-bound form. As a result, Gα guanine nucleotide exchange factor RIC-8 and guanine nucleotide activating protein RGS-7, which have opposing functions, regulate the net movement of the spindle via the Gα protein complex [82, 96, 97]. Modulation of the cortical forces are also mediated in part by phosphorylation of LIN-5 by the atypical protein kinase C PKC-3 [98]. An additional regulation of spindle activity is realised by Gβγ, which binds to excess Gα-GDP [81].

The composition of the membrane lipids has also been found to modulate the amount of *C. elegans* ternary complex on the cortex via the activity of casein kinase I (Csnk1). Increased Csnk1 reduces the amount of kinase PPK-1, which catalyses the production of PIP_2 [99]. Csnk1 also has an inhibitory effect on the amount of GPR-1/2 and LIN-5 at the cortex: depletion of Csnk1 leads to their increased presence of GPR-1/2 and LIN-5 on the cortex, leading to increased spindle movements [99]. Whether these dual functions of Csnk1 are independent is uncertain. The specific role of PIP_2 in the anchoring of GPR-1/2 and LIN-5 remains to be discovered.

Spindle positioning and orientation in human cells is dependent on the ternary complex

Interphase cells in *H. sapiens* have a centrally located nucleus and centrosome. By local perturbation of microtubules on one side of the cell, it was shown that this centring required the activity of cortically localised dynein, which presumably produces pulling forces on the radial microtubule array of the interphase cell [100]. During mitosis, similar dynein activity at the cell cortex is essential for centring of the mitotic spindle in metaphase. The conserved ternary complex of NuMA/LGN/Gαi is responsible for the cortical anchoring of dynein in this system, without which spindle positioning and orientation are defective [84, 101] (Fig. 4C).

During metaphase, negative regulation of NuMA is achieved by its phosphorylation by CDK1/cyclin B [102], which in turn reduces dynein localisation at the membrane. In contrast, the NuMA phosphatase PPP2CA enhances the membrane localisation of the ternary complex-dynein [102]. Dual regulation by the kinase and the phosphatase of NuMA is proposed to be required for ensuring that there are no exaggerated spindle movements [102]. The localisation pattern of cortical dynein during metaphase shows an exclusion from the equatorial region of the cell. This pattern results from negative regulation via Ran-GTP produced around the chromosomes and is essential for spindle positioning [103].

NuMA's association with phosphoinositides is required during anaphase in human cells

Cortical dynein is required for spindle elongation in *H. sapiens* during anaphase. However, the targeting of dynein to the anaphase membrane is independent of LGN/Gαi [104]. Here, the phosphoinositde-binding ability of NuMA is sufficient for cortical localisation of NuMA-dynein. CDK1 activity is lowered during anaphase, and in turn, the population of non-phosphorylated NuMA increases [103–105]. Indeed, by inactivating CDK1 during metaphase, Kotak et al. were able to demonstrate an increase in the cortical localisation of dynein, similar to that seen in anaphase. In vitro, NuMA binds to an array of phosphoinositides, with highest affinity for phosphatidyl inositol 4-phosphate (PIP) and PIP_2 [104, 106]. The association of NuMA with membrane polyanionic phospholipids is restricted to the poles of the cell by the centralspindlin complex proteins CYPK1 and MPLK1 [104], although the precise mechanism of inhibition at the equatorial region is still unknown. Even though NuMA could interact with PIP during metaphase, low levels of non-phosphorylated NuMA or PIP/PIP_2 or higher affinity for LGN/Gαi are proposed to act as a deterrent for direct binding to the membrane via phospholipids [104]. The similarity between NuMA and LIN-5 in their binding to membrane PIP_2 could be an evolutionarily conserved mechanism. Whether such binding is present in the *Drosophila* ortholog Mud remains to be uncovered.

Does dynein's association with the ternary complex regulate its activity?

Dynein that is bound to the membrane via the *Drosophila* Mud/Pins/Gα complex is thought to produce the pulling force required to move the spindle pole towards the apical region of the neuroblast. The dynein regulators Lis1 and dynactin are essential in this process, because mutants of Lis1 or dynactin show reduced movement of the spindle towards the apical region [107]. Any defect in the proteins required for asymmetric positioning of the spindle gives rise to excess production of neural stem cells, reduced differentiated cells and lethality [108]. Although the core system required for the asymmetric cell division in neuroblasts has been identified, there are still many questions regarding the precise mechanism of the process and its interaction with a parallel pathway, which involves Pins and kinesin heavy chain 73 (Khc73). Khc73 is thought also to act as a tether for the microtubule in the apical cortex of neuroblasts [88], and may serve to deliver components of the dynein-Pins-Mud pathway to the plus-end of microtubules. Future experiments with mutants of the components involved in both pathways will be required to delineate spatial and temporal distinctions in the functioning of the dynein-Pins-Mud pathway and the Khc7-mediated pathway in apical polarization of the spindle.

In *C. elegans*, association between the ternary complex of LIN-5/GPR-1/2/Gα and dynein is required for the asymmetric posterior positioning of the spindle [72]. The N-terminus of the ternary complex protein LIN-5 binds to

dynein [101], but the subunit that mediates the binding is not yet known. Reduction in levels of any of the three ternary complex proteins depletes levels of dynein at the cortex and consequently reduces the amount of spindle movement [109]. Conversely, increase in the level GPR-1/2 at the cortex leads to increased cortical dynein localisation and increased spindle movements. Asymmetric localisation of polarity cues to the posterior of the cell leads to preferential localisation of the ternary complex-bound dynein at the posterior edge, which subsequently results in net movement of the spindle towards the posterior [92].

As was proposed in *A. nidulans*, the role of dynein in spindle positioning in *C. elegans* could be one of two possibilities – (i) as a tether that binds to the depolymerising plus-ends of the microtubule emanating from the spindle, thereby translating the shrinkage of the microtubules to a pulling force [71] or, (ii) as a minus-end-directed motor that produces the pulling force for moving the spindle [110]. Dynein's role in moving the spindle was postulated based on experiments where partial depletion of dynein HC or $p150^{Glued}$ subunit of dynactin resulted in improper orientation of the spindle [111]. The dynein regulator, Lis1 was also demonstrated to be required for dynein-mediated pulling of astral microtubules to the posterior of the cell [112]. Whether dynein's activity is regulated upon binding to LIN-5 is unknown.

The subunit of dynein that interacts with mammalian NuMA has not been identified. In the polarised MDCK epithelial cells, Lis1 was proposed to be required for the proper attachment of astral microtubules to the cortex via the dynein complex. In cells overexpressing Lis1, in addition to spindle misorientation, distribution of dynein-dynactin at the cortex was perturbed [113]. However, the precise function of dynein regulators in this process is unknown.

While studies on dynein regulation by anchor binding have emerged from recent work in yeast anchor proteins, much is yet to be discovered about their ternary complex counterparts. Does dynein also undergo a switch in function from inactive to active upon cortical localisation via the ternary complexes?

What could be the mechanism by which dynein is regulated in these systems?

Torisawa et al. [29] suggest an auto-inhibited default state of single dynein, where the dynein heads are 'stacked' together. Upon binding to cargo in a cluster of motors, this inhibition is proposed to be relieved as the motor heads become separated. Additionally, the interaction of dynactin subunit $p150^{Glued}$ with dynein HC tail was seen to increase the processivity of dynein [35]. In other studies, dynactin along with the adaptor protein BICD2 were shown to activate dynein motility by binding to dynein HC tail region [6, 16, 27, 28]. In this instance, the dynein tail-dynactin-BICD2 interaction is proposed to introduce an asymmetry between the two tails of the dynein dimer, whereby the auto-inhibition of the single motor suggested in Ref. [29] may be relieved [6]. The activation of dynein by the anchor proteins and complexes may occur in a similar fashion, with the CC domains of Num1, Mcp5, ApsA, Mud, LIN-5 or NuMA acting together with dynactin to cause 'unstacking' of the auto-inhibited dynein dimer.

In vivo single-molecule studies in these systems would enable a huge leap forward in understanding dynein's regulation. Highly illuminated and laminated optical sheet microscopy [114], which was successfully employed in fission yeast to visualise single dynein molecules and its activation upon anchor binding [33], can be implemented with a few minor modifications in other systems where the expression levels are higher and cell architecture is more complex [67]. Single-molecule tracking and subsequent analysis in wild-type and mutant backgrounds of dynein complex components would shed light on the motile state of microtubule- and anchor-bound dynein. In vitro reconstituted systems have been successfully employed in recent work for identification of dynein's (in)activity [25, 27, 28]. Such reconstituted systems featuring the ternary complex and its anchoring of the dynein complex [71] with its accessory proteins would be invaluable to gain insight and compare the modes of regulation of dynein by its anchor at the cortex.

Conclusions and outlook

Anchors of dynein at the cortex are required for proper spindle positioning and orientation or nuclear movement and distribution in organisms ranging from *S. cerevisiae* to *H. sapiens*. It is curious that while in the fungal systems, the task of cortical localisation and dynein binding is performed by a single protein, in metazoans these tasks are distributed to a set of three proteins which make up the ternary complex. Num1, Mcp5 and ApsA, while weakly homologous amongst themselves, share no sequence similarity with any of the ternary complex proteins in *Drosophila*, *C. elegans* or *H. sapiens*. In budding and fission yeast, the anchor protein mediates the activation of dynein and requires the dynein regulator dynactin. In the other systems discussed here, it is unclear whether dynein's activity is regulated in a similar fashion. Recent structural and in vitro studies featuring a complex of dynein-dynactin-BICD2 have given us an insight into how dynein's inherent inactivity is alleviated. It would be interesting to see if there exists a similar mode of regulation of dynein by the anchor proteins and ternary complexes, whereby cortical attachment activates dynein motility. Crystal structures of dynein bound to the anchor/ternary complex will yield an understanding into the type of interaction between the two moieties. Innovative experiments which facilitate observation and perturbation of single dynein motors and their regulators will be required to address how dynein regulation occurs inside the cell. While a lot of knowledge has been accrued about the anchor proteins in fungi and how they regulate dynein motility, several fundamental questions remain unanswered, and will likely form the body of future work in this area of research.

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References

1. **Karki S, Holzbaur EL**. 1999. Cytoplasmic dynein and dynactin in cell division and intracellular transport. *Curr Opin Cell Biol* **11**: 45–53.
2. **Ronald DV**. 2003. The molecular motor toolbox for intracellular transport review. *Cell* **112**: 467–80.
3. **Pavin N, Tolić-Nørrelykke IM**. 2013. Dynein, microtubule and cargo: a ménage à trois. *Biochem Soc Trans* **41**: 1731–5.
4. **Dujardin DL, Vallee RB**. 2002. Dynein at the cortex. *Curr Opin Cell Biol* **14**: 44–9.
5. **Reck-peterson SL, Vale RD, Gennerich A**. 2012. Motile properties of cytoplasmic dynein. *Handb Dynein*, 145–72.
6. **Urnavicius L, Zhang K, Diamant AG, Motz C**, et al. 2015. The structure of the dynactin complex and its interaction with dynein. *Science* **347**: 1441–6.
7. **Reck-Peterson SL, Vale RD**. 2004. Molecular dissection of the roles of nucleotide binding and hydrolysis in dynein's AAA domains in Saccharomyces cerevisiae. *Proc Natl Acad Sci U S A* **101**: 1491–5.
8. **Gibbons IR, Garbarino JE, Tan CE, Reck-peterson SL**, et al. 2005. The affinity of the dynein microtubule-binding domain is modulated by the conformation of its coiled-coil stalk. *J Biol Chem* **280**: 23960–5.
9. **Burgess SA, Walker ML, Sakakibara H, Knight PJ**, et al. 2003. Dynein structure and power stroke. *Nature* **421**: 715–8.
10. **Roberts AJ, Numata N, Walker ML, Kato YS**, et al. 2009. AAA+ ring and linker swing mechanism in the dynein motor. *Cell* **136**: 485–95.
11. **Kon T, Oyama T, Shimo-Kon R, Imamula K**, et al. 2012. The 2.8 Å crystal structure of the dynein motor domain. *Nature* **484**: 345–50.
12. **Schmidt H, Gleave ES, Carter AP**. 2012. Insights into dynein motor domain function from a 3.3-Å crystal structure. *Nat Struct Mol Biol* **19**: 492–7, S1.
13. **Reck-peterson SL, Yildiz A, Carter AP, Gennerich A**, et al. 2006. Single-molecule analysis of dynein processivity and stepping behavior. *Cell* **126**: 335–48.
14. **Karki S, Holzbaur ELF**. 1995. Affinity chromatography demonstrates a direct binding between cytoplasmic dynein and the dynactin complex. *J Biol Chem* **270**: 28806–11.
15. **Vaughan KT**. 1995. Cytoplasmic dynein binds dynactin through a direct interaction between the intermediate chains and p150Glued. *J Cell Biol* **131**: 1507–16.
16. **Chowdhury S, Ketcham SA, Schroer TA, Lander GC**. 2015. Structural organization of the dynein-dynactin complex bound to microtubules. *Nat Struct Mol Biol* **22**: 345–7.
17. **King SJ, Schroer TA**. 2000. Dynactin increases the processivity of the cytoplasmic dynein motor. *Nat Cell Biol* **2**: 20–4.
18. **Huang J, Roberts AJ, Leschziner AE, Reck-Peterson SL**. 2012. Lis1 acts as a "clutch" between the ATPase and microtubule-binding domains of the dynein motor. *Cell* **150**: 975–86.
19. **McKenney RJ, Vershinin M, Kunwar A, Vallee RB**, et al. 2010. LIS1 and NudE induce a persistent dynein force-producing state. *Cell* **141**: 304–14.
20. **Rai A, Rai A, Ramaiya A, Jha R**, et al. 2013. Molecular adaptations allow dynein to generate large collective forces inside cells. *Cell* **152**: 172–82.
21. **Ross JL, Wallace K, Shuman H, Goldman YE**, et al. 2006. Processive bidirectional motion of dynein-dynactin complexes in vitro. *Nat Cell Biol* **8**: 562–70.
22. **Ori-McKenney K, Xu J, Gross S, Vallee R**. 2010. A cytoplasmic dynein tail mutation impairs motor processivity. *Nat Cell Biol* **12**: 1228–34.
23. **Toba S, Watanabe TM, Yamaguchi-okimoto L, Toyoshima YY**, et al. 2006. Overlapping hand-over-hand mechanism of single molecular motility of cytoplasmic dynein. *PNAS* **103**: 5741–45.
24. **Walter WJ, Brenner B, Steffen W**. 2010. Cytoplasmic dynein is not a conventional processive motor. *J Struct Biol* **170**: 266–9.
25. **Trokter M, Mücke N, Surrey T**. 2012. Reconstitution of the human cytoplasmic dynein complex. *PNAS* **109**: 1–6.
26. **Miura M, Matsubara A, Kobayashi T**. 2010. Nucleotide-dependent behavior of single molecules of cytoplasmic dynein on microtubules in vitro. *FEBS Lett* **584**: 2351–5.
27. **Schlager MA, Hoang HT, Urnavicius L, Bullock SL**, et al. 2014. In vitro reconstitution of a highly processive recombinant human dynein complex. *EMBO J* **33**: 1855–68.
28. **McKenney RJ, Huynh W, Tanenbaum ME, Bhabha G**, et al. 2014. Activation of cytoplasmic dynein motility by dynactin-cargo adapter complexes. *Science* **345**: 337–41.
29. **Torisawa T, Ichikawa M, Furuta A, Saito K**, et al. 2014. Autoinhibition and cooperative activation mechanisms of cytoplasmic dynein. *Nat Cell Biol* **16**: 1118–24.
30. **Splinter D, Razafsky DS, Schlager MA, Serra-marques A.** 2012. BICD2, dynactin, and LIS1 cooperate in regulating dynein recruitment to cellular structures. *Mol Biol Cell* **23**: 4226–41.
31. **Soundararajan HC, Bullock SL**. 2014. The influence of dynein processivity control, MAPs, and microtubule ends on directional movement of a localising mRNA. *Elife* **3**: 1–26.
32. **Lammers LG, Markus SM**. 2015. The dynein cortical anchor Num1 activates dynein motility by relieving Pac1/LIS1-mediated inhibition. *J Cell Biol* **211**: 309–322.
33. **Ananthanarayanan V, Schattat M, Vogel SK, Krull A**, et al. 2013. Dynein motion switches from diffusive to directed upon cortical anchoring. *Cell* **153**: 1526–36.
34. **Verhey KJ, Hammond JW**. 2009. Traffic control: regulation of kinesin motors. *Nat Rev Mol Cell Biol* **10**: 765–77.
35. **Tripathy SK, Weil SJ, Chen C, Anand P**, et al. 2014. Autoregulatory mechanism for dynactin control of processive and diffusive dynein transport. *Nat Cell Biol* **16**: 1192–201.
36. **Kormanec J, Schaaff-Gerstenschläger I, Zimmermann FK, Perečko D**, et al. 1991. Nuclear migration in Saccharomyces cerevisiae is controlled by the highly repetitive 313kDa NUM1 protein. *MGG Mol Gen Genet* **230**: 277–787.
37. **Saito TT, Okuzaki D, Nojima H**. 2006. Mcp5, a meiotic cell cortex protein, is required for nuclear movement mediated by dynein and microtubules in fi ssion yeast. *J Cell Biol* **173**: 27–33.
38. **Yamashita A, Yamamoto M**. 2006. Fission yeast Num1p is a cortical factor anchoring dynein and is essential for the horse-tail nuclear movement during meiotic prophase. *Genetics* **1196**: 1187–96.
39. **Fischer R, Timberlake W**. 1995. Aspergillus nidulans apsA (anucleate primary sterigmata) encodes a coiled-coil protein required for nuclear positioning and completion of asexual development. *J Cell Biol* **128**: 485–98.
40. **Farkasovsky M, Küntzel H**. 1995. Yeast Num1p associates with the mother cell cortex during S/G2 phase and affects microtubular functions. *J Cell Biol* **131**: 1003–14.
41. **Adames NR, Cooper JA**. 2000. Microtubule interactions with the cell cortex causing nuclear movements in Saccharomyces cerevisiae. *J Cell Biol* **149**: 863–74.
42. **Segal M, Bloom K**. 2001. Control of spindle polarity and orientation in Saccharomyces cerevisiae. *Trends Cell Biol* **11**: 160–6.
43. **Schuyler SC, Pellman D**. 2001. Search, capture and signal: games microtubules and centrosomes play. *J Cell Sci* **114**: 247–55.
44. **Heil-Chapdelaine RA, Oberle JR, Cooper JA**. 2000. The cortical protein Num1p is essential for dynein-dependent interactions of microtubules with the cortex. *J Cell Biol* **151**: 1337–44.
45. **Farkasovsky M, Küntzel H**. 2001. Cortical Num1p interacts with the dynein intermediate chain Pac11p and cytoplasmic microtubules in budding yeast. *J Cell Biol* **152**: 251–62.
46. **Sheeman B, Carvalho P, Sagot I, Geiser J**, et al. 2003. Determinants of S. cerevisiae dynein localization and activation: implications for the mechanism of spindle positioning. *Curr Biol* **13**: 364–72.
47. **Lee W, Oberle JR, Cooper JA**. 2003. The role of Lissencephaly protein Pac1 during nuclear migration in budding yeast. *J Cell Biol* **160**: 355–64.
48. **Carvalho P, Gupta ML, Hoyt MA, Pellman D.** 2004. Cell cycle control of kinesin-mediated transport of Bik1 (CLIP-170) regulates microtubule stability and dynein activation. *Developmental Cell* **6**: 815–29.
49. **Roberts AJ, Goodman BS, Reck-Peterson SL**. 2014. Reconstitution of dynein transport to the microtubule plus end by kinesin. *Elife* **3**: e02641.
50. **Markus SM, Punch JJ, Lee W**. 2009. Motor- and tail-dependent targeting of dynein to microtubule plus ends and the cell cortex. *Curr Biol* **19**: 196–205.
51. **Markus SM, Lee W**. 2011. Microtubule-dependent path to the cell cortex for cytoplasmic dynein in mitotic spindle orientation. *BipArchitecture* **1**: 209–15.
52. **Yu JW, Mendrola JM, Audhya A, Singh S**, et al. 2004. Genome-wide analysis of membrane targeting by S. cerevisiae pleckstrin homology domains. *Mol Cell* **13**: 677–88.
53. **Tang X, Punch JJ, Lee W-L**. 2009. A CAAX motif can compensate for the PH domain of Num1 for cortical dynein attachment. *Cell Cycle* **8**: 3182–90.

54. **Tang X, St. Germain B, Lee WL**. 2012. A novel patch assembly domain in Num1 mediates dynein anchoring at the cortex during spindle positioning. *J Cell Biol* **196**: 743–56.
55. **Martín-Castellanos C, Blanco M, Rozalén AE, Pérez-Hidalgo L**, et al. 2005. A large-scale screen in S. pombe identifies seven novel genes required for critical meiotic events. *Curr Biol* **15**: 2056–62.
56. **Yamamoto A, West RR, Mcintosh JR, Hiraoka Y**. 1999. A cytoplasmic dynein heavy chain is required for oscillatory nuclear movement of meiotic prophase and efficient meiotic recombination in fission yeast. *J Cell Biol* **145**: 1233–49.
57. **Yamamoto A, Tsutsumi C, Kojima H, Oiwa K**, et al. 2001. Dynamic behavior of microtubules during dynein-dependent nuclear migrations of meiotic prophase in fission yeast. *Mol Biol Cell* **12**: 3933–46.
58. **Vogel SK, Pavin N, Maghelli N, Jülicher F**, et al. 2009. Self-organization of dynein motors generates meiotic nuclear oscillations. *PLoS Biol* **7**: 0918–28.
59. **Niccoli T, Yamashita A, Nurse P, Yamamoto M.** 2004. The p150-Glued Ssm4p regulates microtubular dynamics and nuclear movement in fission yeast. *J Cell Sci* **117**: 5543–56.
60. **Fujita I, Yamashita A, Yamamoto M**. 2015. Dynactin and Num1 cooperate to establish the cortical anchoring of cytoplasmic dynein in S. pombe. *J Cell Sci* **128**: 1555–67.
61. **Zhang J, Han G, Xiang X**. 2002. Cytoplasmic dynein intermediate chain and heavy chain are dependent upon each other for microtubule end localization in Aspergillus nidulans. *Mol Microbiol* **44**: 381–92.
62. **Veith D, Scherr N, Efimov V, Fischer R**. 2005. Role of the spindle-pole-body protein ApsB and the cortex protein ApsA in microtubule organization and nuclear migration in Aspergillus nidulans. *J Cell Sci* **118**: 3705–16.
63. **Efimov VP**. 2003. Roles of NUDE and NUDF proteins of Aspergillus nidulans: insights from intracellular localization and overexpression effects. *Mol Biol Cell* **14**: 871–88.
64. **Tan K, Roberts AJ, Chonofsky M, Egan MJ**, et al. 2014. A microscopy-based screen employing multiplex genome sequencing identifies cargo-specific requirements for dynein velocity. *Mol Biol Cell* **25**: 669–78.
65. **Zhang J, Li S, Fischer R, Xiang X.** 2003. Accumulation of cytoplasmic dynein and dynactin at microtubule plus ends in Aspergillus nidulans is kinesin dependent. **14**: 1479–88.
66. **Lee W, Kaiser MA, Cooper JA**. 2005. The offloading model for dynein function: differential function of motor subunits. *J Cell Biol* **168**: 201–7.
67. **Ananthanarayanan V, Tolić IM**. 2015. Single-molecule imaging of cytoplasmic dynein in vivo. *Method Cell Biol* **125**: 1–2.
68. **Krull A, Steinborn A, Ananthanarayanan V, Ramunno-Johnson D**, et al. 2014. A divide and conquer strategy for the maximum likelihood localization of low intensity objects. *Opt Express* **22**: 210.
69. **Fujita I, Yamashita A, Yamamoto M**. 2010. Contribution of dynein light intermediate and intermediate chains to subcellular localization of the dynein – dynactin motor complex in Schizosaccharomyces pombe. *Genes to Cells* **15**: 359–72.
70. **Hendricks AG, Lazarus JE, Perlson E, Gardner MK**, et al. 2012. Dynein tethers and stabilizes dynamic microtubule plus ends. *Curr Biol* **22**: 632–7.
71. **Laan L, Pavin N, Husson J, Romet-Lemonne G**, et al. 2012. Cortical dynein controls microtubule dynamics to generate pulling forces that position microtubule asters. *Cell* **148**: 502–14.
72. **Kotak S, Gönczy P**. 2013. Mechanisms of spindle positioning: cortical force generators in the limelight. *Curr Opin Cell Biol* **25**: 741–8.
73. **Radulescu AE, Cleveland DW**. 2010. NuMA after 30 years: the matrix revisited. *Trends Cell Biol* **20**: 214–22.
74. **Lu MS, Johnston C a**. 2013. Molecular pathways regulating mitotic spindle orientation in animal cells. *Development* **140**: 1843–56.
75. **Guan Z, Prado A, Melzig J, Heisenberg M,** et al. 2000. Mushroom body defect, a gene involved in the control of neuroblast proliferation in Drosophila, encodes a coiled-coil protein. *Proc Natl Acad Sci U S A* **97**: 8122–7.
76. **Siller KH, Cabernard C, Doe CQ**. 2006. The NuMA-related Mud protein binds Pins and regulates spindle orientation in Drosophila neuroblasts. *Nat Cell Biol* **8**: 594–600.
77. **Lorson MA, Horvitz HR, van den Heuvel S**. 2000. LIN-5 is a novel component of the spindle apparatus required for chromosome segregation and cleavage plane specification in Caenorhabditis elegans. *J Cell Biol* **148**: 73–86.
78. **Lydersen BK, Pettijohn DE**. 1980. Human-specific nuclear protein that associates with the polar region of the mitotic apparatus: distribution in a human/hamster hybrid cell. *Cell* **22**: 489–99.
79. **Gueth-Hallonet C, Weber K, Osborn M**. 1996. NuMA: a bipartite nuclear location signal and other functional properties of the tail domain. *Exp Cell Res* **225**: 207–18.
80. **Yang CH, Lambie EJ, Snyder M**. 1992. NuMA: an unusually long coiled-coil related protein in the mammalian nucleus. *J Cell Biol* **116**: 1303–17.
81. **Gotta M, Ahringer J**. 2001. Distinct roles for Galpha and Gbetagamma in regulating spindle position and orientation in Caenorhabditis elegans embryos. *Nat Cell Biol* **3**: 297–300.
82. **Miller KG, Rand JB**. 2000. A role for RIC-8 (Synembryn) and GOA-1 (Go{alpha}) in regulating a subset of centrosome movements during early embryogenesis in Caenorhabditis elegans. *Genetics* **156**: 1649–60.
83. **Nipper RW, Siller KH, Smith NR, Doe CQ**, et al. 2007. Galphai generates multiple Pins activation states to link cortical polarity and spindle orientation in Drosophila neuroblasts. *Proc Natl Acad Sci U S A* **104**: 14306–11.
84. **Du Q, Macara IG**. 2004. Mammalian Pins is a conformational switch that links NuMA to heterotrimeric G proteins. *Cell* **119**: 503–16.
85. **Du Q, Taylor L, Compton DA, Macara IG**. 2002. LGN blocks the ability of NuMA to bind and stabilize microtubules. A mechanism for mitotic spindle assembly regulation. *Curr Biol* **12**: 1928–33.
86. **Du Q, Stukenberg PT, Macara IG**. 2001. A mammalian Partner of inscuteable binds NuMA and regulates mitotic spindle organization. *Nat Cell Biol* **3**: 1069–75.
87. **Schaefer M, Shevchenko A, Knoblich JA**. 2000. A protein complex containing Inscuteable and the Galpha-binding protein Pins orients asymmetric cell divisions in Drosophila. *Curr Biol* **10**: 353–62.
88. **Siegrist SE, Doe CQ**. 2005. Microtubule-induced Pins/Galphai cortical polarity in Drosophila neuroblasts. *Cell* **123**: 1323–35.
89. **Wee B, Johnston CA, Prehoda KE, Doe CQ**. 2011. Canoe binds RanGTP to promote Pins(TPR)/Mud-mediated spindle orientation. *J Cell Biol* **195**: 369–76.
90. **Wang C, Li S, Januschke J, Rossi F**, et al. 2011. An ana2/ctp/mud complex regulates spindle orientation in Drosophila neuroblasts. *Dev Cell* **21**: 520–33.
91. **Albertson DG**. 1984. Formation of the first cleavage spindle in nematode embryos. *Dev Biol* **101**: 61–72.
92. **Grill SW, Gönczy P, Stelzer EH, Hyman AA**. 2001. Polarity controls forces governing asymmetric spindle positioning in the Caenorhabditis elegans embryo. *Nature* **409**: 630–3.
93. **Grill SW, Howard J, Schäffer E, Stelzer EHK**, et al. 2003. The distribution of active force generators controls mitotic spindle position. *Science* **301**: 518–21.
94. **Grill SW, Hyman AA**. 2005. Spindle positioning by cortical pulling forces. *Dev Cell* **8**: 461–5.
95. **Skop AR, White JG**. 1998. The dynactin complex is required for cleavage plane specification in early Caenorhabditis elegans embryos. *Curr Biol* **8**: 1110–6.
96. **Afshar K, Willard FS, Colombo K, Johnston CA**, et al. 2004. RIC-8 is required for GPR-1/2-dependent Galpha function during asymmetric division of C. elegans embryos. *Cell* **119**: 219–30.
97. **Hess HA, Röper J-C, Grill SW, Koelle MR**. 2004. RGS-7 completes a receptor-independent heterotrimeric G protein cycle to asymmetrically regulate mitotic spindle positioning in C. elegans. *Cell* **119**: 209–18.
98. **Galli M, Muñoz J, Portegijs V, Boxem M**, et al. 2011. APKC phosphorylates NuMA-related LIN-5 to position the mitotic spindle during asymmetric division. *Nat Cell Biol* **13**: 1132–8.
99. **Panbianco C, Weinkove D, Zanin E, Jones D**, et al. 2008. A casein kinase 1 and PAR proteins regulate asymmetry of a PIP(2) synthesis enzyme for asymmetric spindle positioning. *Dev Cell* **15**: 198–208.
100. **Burakov a**. 2003. Centrosome positioning in interphase cells. *J Cell Biol* **162**: 963–9.
101. **Kotak S, Busso C, Gönczy P**. 2012. Cortical dynein is critical for proper spindle positioning in human cells. *J Cell Biol* **199**: 97–110.
102. **Kotak S, Busso C, Gönczy P**. 2013. NuMA phosphorylation by CDK1 couples mitotic progression with cortical dynein function. *EMBO J* **32**: 2517–29.
103. **Kiyomitsu T, Cheeseman IM**. 2012. Chromosome- and spindle-pole-derived signals generate an intrinsic code for spindle position and orientation. *Nat Cell Biol* **14**: 311–7.
104. **Kotak S, Busso C, Gönczy P**. 2014. NuMA interacts with phosphoinositides and links the mitotic spindle with the plasma membrane. *EMBO J* **33**: 1–6.

105. **Collins ES, Balchand SK, Faraci JL, Wadsworth P**, et al. 2012. Cell cycle-regulated cortical dynein/dynactin promotes symmetric cell division by differential pole motion in anaphase. *Mol Biol Cell* **23**: 3380–90.
106. **Zheng Z, Wan Q, Meixiong G, Du Q**. 2014. Cell cycle-regulated membrane binding of NuMA contributes to efficient anaphase chromosome separation. *Mol Biol Cell* **25**: 606–19.
107. **Siller KH, Doe CQ**. 2008. Lis1/dynactin regulates metaphase spindle orientation in Drosophila neuroblasts. *Dev Biol* **319**: 1–9.
108. **Cabernard C, Doe CQ**. 2009. Apical/basal spindle orientation is required for neuroblast homeostasis and neuronal differentiation in Drosophila. *Dev Cell* **17**: 134–41.
109. **Srinivasan DG, Fisk RM, Xu H, van den Heuvel S**. 2003. A complex of LIN-5 and GPR proteins regulates G protein signaling and spindle function in C. elegans. *Genes Dev* **17**: 1225–39.
110. **Kozlowski C, Srayko M, Nedelec F**. 2007. Cortical microtubule contacts position the spindle in C. elegans embryos. *Cell* **129**: 499–510.
111. **Severson AF, Bowerman B**. 2003. Myosin and the PAR proteins polarize microfilament-dependent forces that shape and position mitotic spindles in Caenorhabditis elegans. *J Cell Biol* **161**: 21–6.
112. **Nguyen-Ngoc T, Afshar K, Gönczy P**. 2007. Coupling of cortical dynein and G alpha proteins mediates spindle positioning in Caenorhabditis elegans. *Nat Cell Biol* **9**: 1294–302.
113. **Faulkner NE, Dujardin DL, Tai CY, Vaughan KT**, et al. 2000. A role for the lissencephaly gene LIS1 in mitosis and cytoplasmic dynein function. *Nat Cell Biol* **2**: 784–91.
114. **Tokunaga M, Imamoto N, Sakata-Sogawa K**. 2008. Highly inclined thin illumination enables clear single-molecule imaging in cells. *Nat Method* **5**: 159–61.