Molecular motors

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New pieces for the Lis1–dynein puzzle

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The microtubule motor dynein is regulated by lissencephaly-1 (Lis1) at several points during its complex activation process. Two papers reveal the molecular mechanism for two steps: the beginning, when Lis1 acts as a wedge to disrupt dynein's autoinhibited conformation; and the end, when microtubule binding ejects Lis1 from the motor.

Lis1 is a key causative protein in lissencephaly, a neurodevelopmental disorder in which the brain develops without its characteristic folds. At the molecular level, Lis1 (and its homolog in yeast, Pac1; collectively referred to here as Lis1) regulates the cytoplasmic dynein-1 motor (dynein), which transports various cargoes along microtubules throughout the cell. Lis1 is thought to have several effects on dynein (reviewed in Markus et al.[1]). One of its major roles is to activate dynein at distinct points during the motor's activation process. First, Lis1 acts to overcome dynein autoinhibition. It then separately promotes the formation of the multiprotein motile complex, where dynein binds to coactivator dynactin and a cargo adaptor to link the complex to cargo (Fig. 1). However, despite the progress in deciphering the regulatory activity of Lis1, there are still mechanistic gaps. Two papers in this issue of *Nature Structural & Molecular Biology* reveal information about the action of Lis1 at two points along the dynein activation pathway. Karasmanis, Reimer, Kendrick et al.[2] report an unanticipated feature of Lis1's interaction with dynein alone, while Ton, Wang, Chai et al.[3] answer the longstanding question of how Lis1 dissociates from dynein after activating the dynein–dynactin–adaptor complex.

In the first highlighted study, Karasmanis et al.[2] focused on the interaction of Lis1 with dynein before it forms the motile complex. Dynein alone is locked in an autoinhibited conformation known as phi (Φ)[4]. In this conformation, the two motor domains are bound together with the microtubule-binding domains crossed, unable to productively bind to the microtubule. Electron microscopy (EM) structures have shown that Lis1 binds directly to the dynein motor in a manner that is incompatible with the phi conformation[5–7]. This led to a model whereby dynein stochastically switches from the autoinhibited phi to an open conformation, with Lis1 binding biasing dynein toward the open, activatable conformation. Here, Karasmanis et al.[2] identified a minor subset of yeast Lis1–dynein complexes from their previous dataset[7] that are in a fascinating conformation. In this state, which the authors named chi (X, for the letter that follows Φ in the Greek alphabet), two Lis1 dimers are sandwiched between two dynein motors domains. The authors found a unique interaction interface between Lis1 and dynein in this conformation (here referred to as the chi interface) and

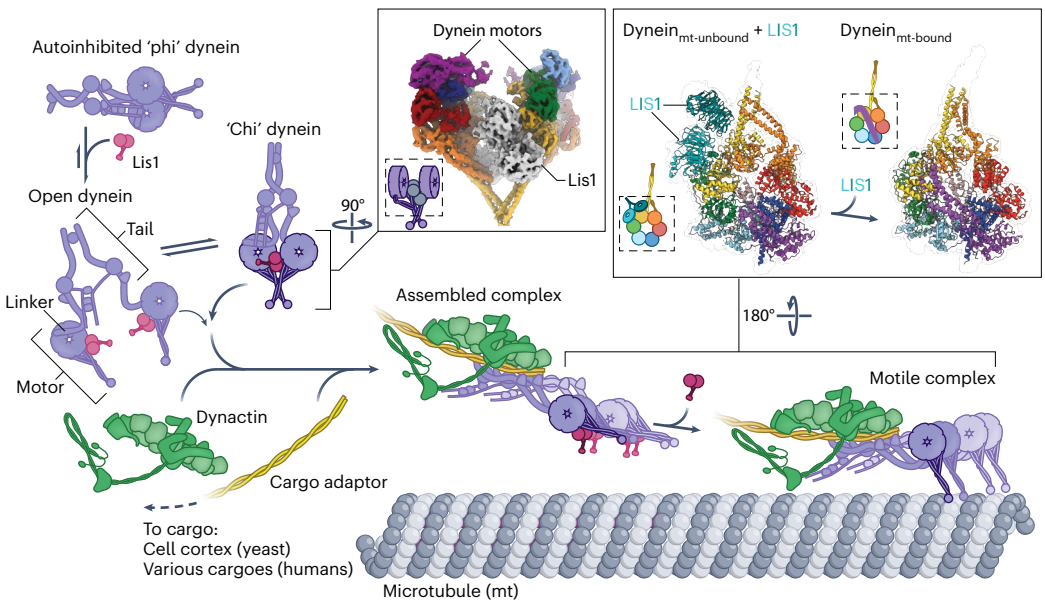

Fig. 1 | Schematic showing Lis1 activation of dynein. Dynein alone is autoinhibited in the phi conformation. During a stochastic opening event, Lis1 binds and prevents dynein from reclosing. Once bound to Lis1, dynein can form the chi conformation, with two Lis1 dimers sandwiched between the two dynein motor domains. This intermediate is speculated to facilitate the binding of dynein to dynactin and the cargo adaptor, which recruits the complex to cargo. This assembled complex can then bind the microtubule, causing the dissociation of Lis1 and movement of the cargo towards the microtubule minus end. Figures adapted from refs. 2,3, Springer Nature Limited.

investigated whether these interactions are important to Lis1-mediated dynein regulation.

Mutating the amino acids that make up the chi interface phenocopied dynein deletion in yeast, which prevents nuclei from segregating properly during mitosis. This phenotype can be rescued by introducing residues that disrupt the autoinhibited phi conformation, which suggests that the chi conformation follows phi during the dynein activation process. The authors also mutated the chi interface residues on human Lis1 to examine their importance using in vitro total internal reflection fluorescence (TIRF) microscopy motility assays with human proteins. These mutations reduced the ability of Lis1 to increase the number of motile dynein complexes. Together, these experiments suggest that the interface that Lis1 makes with dynein in the chi conformation is important for the function of Lis1 in both yeast and humans.

The authors[2] proposed that the chi conformation is an intermediate between phi and the assembled complex, priming the dynein to bind dynactin and activating adaptor proteins. Key to this model would be determining the conformation of the dynein tails when dynein is bound to Lis1 in the chi state. In the autoinhibited phi state, the tails are twisted[4]. These tails untwist when dynein transitions to open state, but negative stain EM data shows them to be flexible, adopting a range of conformations[4]. To bind to dynactin and form a motile complex, the tails must adopt a parallel configuration to bind adjacent grooves on dynactin[4]. The authors proposed that in the chi conformation, the dynein tails are in a semiparallel state, more rigid than in the open state, and hence are primed to bind to dynactin.

By identifying and showing the importance of the chi interface in vitro and in vivo, this study paves the way for future investigations into this conformation. Given the low proportion of chi observed in the EM studies, a clear question is how much of this conformation exists in cells. Perhaps chi is a transient intermediate, or it is stabilized in cells by other factors. Answering this question will be important in establishing the order of events during dynein activation.

In the second highlighted study, Ton et al.[3] investigated the conformational changes that occur when Lis1 is bound to the dynein motor domain. This is particularly pertinent to the question of when and how Lis1 dissociates from the dynein–dynactin–adaptor complex. Most data suggest that Lis1 predominantly dissociates from the moving complex[6,8–10]. However, some studies have found that Lis1 can remain attached[9,11,12], particularly in certain nucleotide states of the ATP-hydrolyzing dynein motor domain[13]. It has been postulated that this occurs under high load, which could represent an alternative mode of Lis1 regulation[12,13].

Here, the authors[3] first generated constructs of the dynein motor locked in its two major conformations: microtubule-bound and microtubule-unbound. They then examined the binding of Lis1 to these constructs, showing that Lis1 binds stronger to the microtubule-unbound state. The authors also found that nucleotide analogs that bias dynein to a microtubule-unbound state also increase Lis1 binding. These analogs had little effect on the microtubule-bound construct, suggesting that the microtubule binding state is the primary determinant of Lis1 binding. Interestingly, in the absence of nucleotide, Lis1 bound similarly to both microtubule-bound and microtubule-unbound dynein, as has been found in previous experiments[13].

Ton et al. also solved three high-resolution cryo-EM structures of human dynein motor: one in the microtubule-unbound state in complex with Lis1 (similar to one reported by Reimer et al.[14]), one in the microtubule-unbound state without Lis1 and one in the microtubule-bound state. Comparing the microtubule-unbound structures showed that Lis1 binding does not significantly alter the conformation of the dynein motor domain. In the microtubule-bound state, the authors found distortion of one of the two Lis1-binding sites that would weaken Lis1 binding, explaining the lower affinity of this state for Lis1. The authors concluded that dynein's conformation affects its ability to bind Lis1, but Lis1 binding does not affect dynein's conformation and hence its mechanochemical cycle. Finally, they examined the contributions of the two Lis1-binding sites on the dynein motor (not including the chi interface). Their experiments showed that both Lis1-binding sites on dynein, site$_{ring}$ and site$_{stalk}$, are important in cells, which is similar to results from previous work[7]. However, the authors found that in vitro, site$_{ring}$ forms a stronger interaction, as mutating this site, but not site$_{stalk}$, abolishes Lis1 binding.

Together, these experiments strongly suggest that the binding of the dynein–dynactin–cargo complex to the microtubule generally results in the dissociation of Lis1, explaining why previous studies mostly showed a low percentage of Lis1 co-migrating with these complexes as they move along microtubules. These data are also consistent with Lis1 acting as a steric block to prevent open dynein from switching back into its autoinhibited state. The conclusion that Lis1 does not affect the dynein motor's mechanochemical cycle is less concrete, considering the opposing studies as to whether Lis1 can alter this activity[5,12,13,15]. Indeed, previous reports point to Lis1 binding regulating dynein's ATPase cycle via the linker arm rather than via the motor domain itself[15]. Further experiments will be required to resolve these opposing models.

Overall, the two studies by Karasmanis et al.[2] and Ton et al.[3] provide mechanistic insight into the complex pathway of Lis1 activation and raise questions to be addressed in future studies. One question is: Given that Lis1 dissociates from dynein if the motor binds to the microtubule, how does Lis1 stay bound to open dynein to then assist with forming a complex with dynactin and adaptor? Perhaps the chi conformation could keep dynein from binding the microtubule, like the autoinhibited phi state[4]. A second major question is: What is the significance of the alternative model of Lis1-mediated dynein regulation, which suggests that Lis1 binding to dynein can form a high-affinity microtubule-bound state under high load? Answering these questions will be key to untangle the regulatory mechanism of Lis1 and to understand how its mutation contributes to lissencephaly.

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Competing interests
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