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Competing interests

The authors declare no competing interests.

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LIS1 cracks open dynein

Active transport along microtubules by molecular motors is a crucial cellular process that is disrupted in human diseases. Single-molecule studies from three independent groups reveal a new molecular mechanism for how cells control the activity of the complex microtubule motor cytoplasmic dynein via the neurodevelopmental protein LIS1.

Richard J. McKenney

Microtubule-based transport by kinesin and dynein family motor proteins is crucial for a large variety of cellular functions. In the developing vertebrate nervous system, these motors play critical roles related to cell division, polarisation, and migration. Lissencephaly ('smooth brain') is a severe neurodevelopmental disorder caused by mutation or deletion of the LIS1 gene. LIS1 was identified as a cytoplasmic dynein 1 (hereafter 'dynein') regulatory factor nearly twenty years ago, focusing attempts to understand what roles this protein plays in the dynein transport system. Despite efforts from several groups to elucidate the molecular mechanisms of LIS1's effects on dynein, its precise functions have remained mysterious and controversial. Recent advances in our understanding of how dynein is autoinhibited[1] and activated[2,3] have yielded new tools and assays with which to revisit the role of LIS1 in these recently uncovered phenomena. In this issue of *Nature Cell Biology*, three groups have independently uncovered important new insights into the long-sought LIS1 mystery[4–6]. Using complementary biochemical, biophysical, and cell biological approaches, as well as supporting data from ref. [7], they provide compelling evidence that the role of LIS1 is to favour the release of dynein's autoinhibited state and facilitate the formation of activated, motile dynein–dynactin–adapter complexes.

Since its isolation nearly 35 years ago, dynein was thought to be constitutively active on the basis of its robust activity in multimotor microtubule gliding and enzymatic assays. This assumption raised questions about the role of dynein's ubiquitous co-factor complex dynactin, which is required for most, if not all, of dynein's myriad of cellular functions. Further, it was a mystery in the field as to why no biochemically stable co-complex of dynein and dynactin could be isolated and characterised. In 2014, two reports[2,3] revealed that isolated mammalian dynein (unlike isolated yeast dynein[8]), was in fact not competent to move processively along microtubules on its own. Rather, a set of cargo-specific adapter molecules is necessary to mediate a direct interaction between dynein and dynactin, resulting in the activation of processive motor activity. These studies revealed a fundamental characteristic of dynein that was previously missed in the prior decades

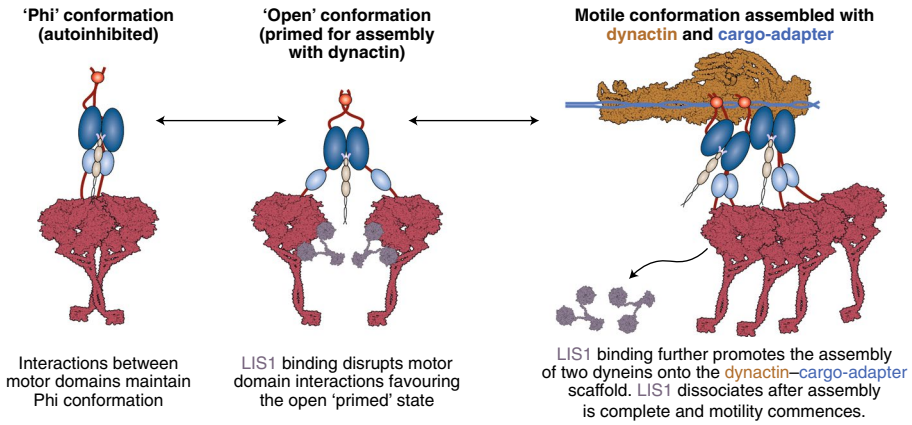

Fig. 1 | Model for LIS1 function in the assembly of activated dynein–dynactin–cargo-adapter complexes. The three studies published here[4–6] provide evidence that LIS1 binding to the motor domains of dynein may bias this conformational equilibrium towards the 'open' state, facilitating the next conformational transition in the presence of dynactin and a cargo-specific adapter molecule. LIS1 further facilitates the loading of two dynein dimers onto a single dynactin scaffold in the presence of various types of cargo-adapter molecules. How it does so remains to be determined, but it may be a consequence of simply holding the dynein in the open state. Loading onto dynactin reconfigures the tail domains of dynein, allowing reorientation of the motor domains to a parallel conformation that is conducive for processive transport along the microtubule. LIS1 does not need to remain bound to the dynein motor domains once this transport-component configuration is achieved. In this way, LIS1 acts as a catalytic assembly factor for the activation of the dynein motor.

of work: dynein requires a coordinated series of structural changes and extrinsic factors to unlock its inherent motor capability.

The basis for these observations was recently revealed via high-resolution cryo-electron microscopy (cryo-EM) and single-molecule work[1]. Early EM work revealed a peculiar structural conformation of isolated dynein termed the 'Phi particle' for its resemblance to the Greek letter[9]. Cryo-EM recently revealed that interactions between the two motor domains within the dynein dimer underlie the Phi conformation[1], leading to autoinhibition of the motor[1] (Fig. 1). The Phi conformation is presumed to be in some equilibrium with a second state termed 'open', in which the interface between the two motor domains is broken, but the motor tail remains twisted in such a way as to prevent parallel arrangement of the motor domains necessary for movement. Notably, the Phi conformation has since been observed in situ for cytoplasmic dynein-2 (ref. [10]), which drives intraflagellar transport within cilia. The culmination of this work revealed that large structural rearrangements of the dynein dimer must occur to facilitate processive movement along microtubules. The interactions between the dynein motor domains that underpin the Phi conformation must be broken, and the symmetrically twisted tail domains must be rearranged to allow parallel binding along the dynactin Arp filament to activate motor activity. The new data presented in the current manuscripts strongly argue for a role for LIS1 in mediating the transition between the autoinhibited Phi conformation and dynein's active conformation.

Studies of the yeast homologs of dynein and LIS1 (Pac1) had previously characterised a LIS1 binding site on the dynein AAA+ motor[11], and Htet et al.[4] confirmed that binding site on mammalian dynein. All three studies capitalised on one important clue: LIS1 binding to this site is sterically incompatible with the Phi particle conformation. With this observation in hand, all three studies utilised in vitro reconstitution with purified components to test the model that LIS1 binding may destabilise the Phi conformation to facilitate reorganisation of the autoinhibited dynein dimer into an active conformation. Whereas the work from Elshenawy et al.[6] and Htet et al.[4] focused on mammalian proteins, Marzo et al.[5] utilised the well-studied dynein system from budding yeast. An immediately surprising finding from this work was that yeast dynein, like its mammalian counterpart, also adopts the Phi conformation in solution. Here, once again, the field had assumed that yeast dynein was constitutively active due to its robust movement in single-molecule assays, yet Marzo et al.[5] revealed that this activity is in fact submaximal. Utilising mutations that disrupt the Phi particle interface, Marzo et al.[5] observed substantially increased run lengths, implying that the motor may spontaneously transition to this conformation, prematurely terminating its movement. Notably, a similar enhancement of run length was also facilitated by the presence of purified Pac1/LIS1, revealing that Pac1/LIS1 is likely capable of destabilising the Phi conformation, favouring the more active form of yeast dynein. Marzo et al.[5] nicely followed up these observations with cell biological experiments demonstrating that mutations that disrupt the Phi conformation partially bypass the requirement of Pac1/LIS1 for proper dynein localisation and function in cells (similar results were recently reported in *Aspergillus*[7]). Finally, Marzo et al.[5] found that previously reported inhibitory effects of Pac1/LIS1 on yeast dynein motility at least partially resulted from spurious binding of Pac1/LIS1 to microtubules. One interesting facet of the yeast system is that dynein's association with dynactin is not required for robust motility in vitro, despite the fact that dynactin is critical for dynein's functions in yeast cells. Future experiments focusing on the effects of dynactin and putative cargo-adapter molecules with yeast dynein and Pac1/LIS1 will no doubt shed further light on this intriguingly distinct dynein system.

Elshenawy et al.[6] and Htet et al.[4] each focused on the mammalian dynein system in which cargo-adapter molecules must link dynein to dynactin to activate processive movement. Both groups found that addition of LIS1 increased the velocity of dynein–dynactin–cargo-adapter complexes, in agreement with previous results[12,13]. Elshenawy et al.[6] nicely demonstrated that this effect comes from an increased stepping rate, but not a change in step size, of the motile dynein complexes. Dynein–dynactin complexes also displayed higher net forces in the presence of LIS1, allowing them to more effectively compete against kinesin motors. One interesting facet of the mammalian system is that distinct cargo-adapter molecules predominantly recruit one dynein dimer per dynactin instead of two[14] (Fig. 1). Htet et al.[4] performed a heroic examination of many of the reported dynein cargo-adapters and found that the presence of LIS1 increased the velocity and percentage of processive complexes for all of the examined cargo-adapters. Both groups then utilised different cargo-adapter molecules, or cleverly, truncated dynein tail constructs that prevent the recruitment of a second active motor, to demonstrate that this effect is mediated by LIS1, favouring the assembly of two dynein dimers onto a single dynactin. To drive this point home, both groups utilised multicolour imaging of differentially labelled dynein to unambiguously visualise the effect of LIS1 in promoting the assembly of motile complexes containing two dynein dimers. Thus, LIS1 plays multiple roles in the ordered assembly of dynein–dynactin–cargo-adapter complexes.

All these data are consistent with the model that LIS1 promotes the release of dynein from the Phi conformation to assemble active dynein–dynactin complexes but point to a further role for LIS1 in helping load a second dynein onto these activated complexes (Fig. 1). How LIS1 mediates this effect remains unclear, but a direct interaction between a LIS1 molecule and adjacent motor domains from different dynein dimers is an intriguing possibility. As LIS1 is itself a dimer, Htet et al.[4] surprisingly found that a monomeric LIS1 construct was still capable of enhancing the velocity of dynein–dynactin complexes, a readout of the number of dyneins bound to dynactin, leaving this question open for future studies. Finally, all three groups asked whether LIS1 remained bound to the activated dynein or dynein–dynactin–cargo-adapter complexes during movement. In the yeast system, Marzo et al.[5] observed robust co-localisation of Pac1/LIS1 with moving dynein molecules, though velocity was reduced compared to that of motors without bound LIS1. In the mammalian system, although a fraction of activated motor complexes did retain LIS1 bound, these moved at modestly lower velocities than those that did not retain LIS1, an apparent contradiction to earlier reports[12,13]. The basis for this lower velocity remains to be determined, but both groups concluded that LIS1 does not generally need to remain bound to mammalian dynein after assembly of the activated complexes, thus acting like a true catalytic assembly factor.

Together, these studies make a powerful argument for the role of LIS1 as a general dynein activation factor, in line with much of the previous genetic and cell biological data. Still, many unanswered questions remain. Foremost among them is a formal demonstration that LIS1 does indeed bias the conformation of dynein away from the autoinhibited Phi form. This should be straightforward with modern EM approaches. Second, the

data argue for a general role of LIS1 in dynein activation, which raises the question of why the Lissencephaly phenotype predominantly affects developing neurons, despite widespread expression of both dynein and LIS1. Finally, LIS1 functions with its paralogous binding partners NdEL1 and NdE1 in cells[15]. Their roles in the activation pathway uncovered by these studies will be of paramount interest to the field in the near future. After nearly twenty years of intense effort, the mystery of LIS1 is finally beginning to crack, revealing many more surprises than those of us in the field could have anticipated. The new models proposed by these elegant studies will no doubt keep us busy for the next twenty years as well! ❐

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Competing interests

The author declares no competing interests.