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Understanding the Roles of Nudel/Lis1/Dynein Pathway in Cell Motility*

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Under the support of multiple grants by NSFC, including General Program, Key Program, National Science Fund for Distinguished Young Scholars, and Fund for Creative Research Groups, the research group explored how the Nudel/Lis1/dynein pathway functions in cell motility. Cytoplasmic dynein is a huge protein complex which, like cars, can use microtubules (MTs) as "highways" to transport "cargos" by utilizing ATP as the energy supply. Dynein is widely involved in cell activities requiring MT-based motility, for instances, membrane/protein trafficking and mitosis, and migration. Dynein function requires another accessory protein complex, dynactin. Dynactin appears important for localizing dynein to certain target sites[1,2]. How dynein functions are regulated, however, is poorly known.

The group initially worked on a protein termed mitosin or CENP-F. Mitosin is a kinetochore protein in M phase, thus presumably involved in mitosis[3]. In a yeast two hybrid screen followed by bioinformatics analysis, we identified two homologous novel proteins in human cells, tentatively termed Mitap1 (for mitosin-associated protein 1) and Mitap1r (for Mitap1-related protein), as proteins associated with mitosin. We found that Mitap1 exhibited centrosome and spindle localization and, like mitosin, is highly phosphorylated in M phase. At the end of 2000, three papers in Neuron reported existence of an evolutionarily-conserved dynein pathway, in which Lis1, murine NudE, and a NudE-like human protein, Nudel, are potential dynein regulators in neuronal cell migration through direct interactions with dynein heavy chain (DHC). While haplo-insufficiency of Lis1 has previously been shown to impair neuronal migration during the development of the central nervous system, leading to type I lissencephaly, a severe congenital disease characteristic of smooth brain surface, mental retardation, and short life span, both NudE and Nudel are proteins poorly studied[4,5]. We found that NudE happened to be an ortholog of Mitap1r, whereas Nudel is identical to Mitap1. To clarify whether Nudel/NudE and their interaction with Lis1 has a role in dynein function in M phase, we created a Nudel mutant lacking the Lis1-binding activity. Indeed, when over expressed, this mutant, NudelN20, impaired dynein-mediated transport of kinetochore proteins to spindle poles along the spindle[6,7], a process important for inactivation of the spindle checkpoint, which guarantees proper timing of anaphase onset[8].

To further understand the interplay among Nudel, Lis1 and dynein, we created another mutant, NudelC36, to selectively disrupt the Nudel-DHC interaction. We found that over expression of either NudelN20 or NudelC36 resulted in dispersions of the membranous organelles whose trafficking depend on dynein. Nevertheless, over expression of either the wild-type Nudel or a double mutant NudelN20/C36 had little effect. Time-lapse microscopy confirmed significant reduction in both the frequency and velocity of the minus end-directed motions of lysosomes[9]. Therefore, we concluded that both the Nudel-Lis1 interaction and the Nudel-DHC interaction are crucial for dynein activity (Figure 1)[9]. RNA interference (RNAi)-mediated silencing of Nudel expression also resulted in Golgi apparatus fragmentation, suggesting an essential role of Nudel in dynein-mediated membrane trafficking (Figure 1)[9]. In addition, we found that Nudel is also required for dynein-mediated transport of centrosome proteins (Figure 2)[10]. Therefore, Nudel appears to serve as a general regulator of dynein.

When analyzing Nudel functions at the centrosome, we found that Nudel associated with the mother

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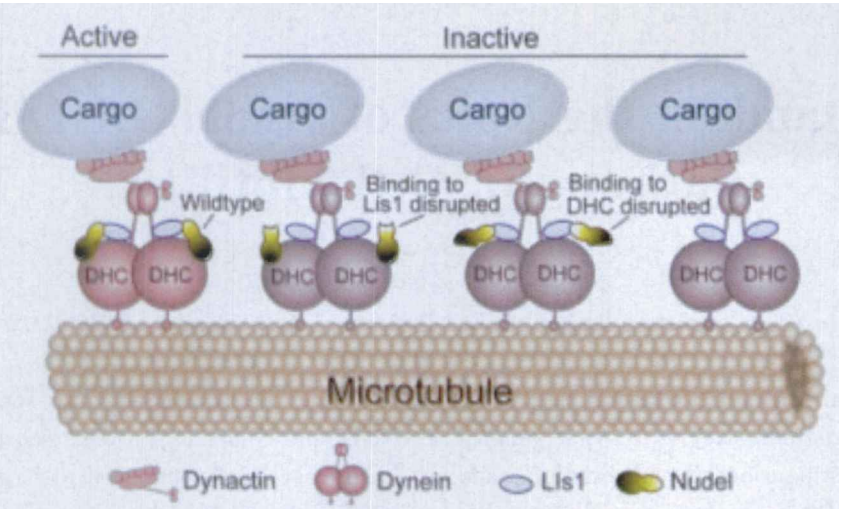

Figure 1 Active dynein requires Nudel and its interactions with both Lis1 and DHC

Disrupting either interaction or silencing Nudel expression impairs dynein activity[9].

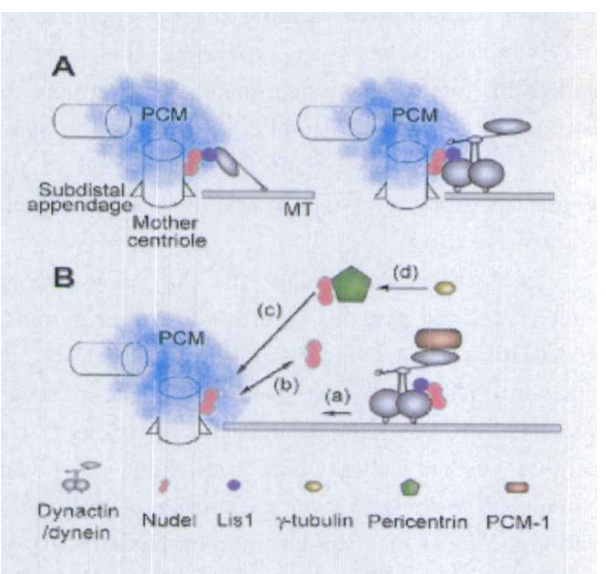

Figure 2 Models delineating roles of Nudel at the centrosome[10]

(A) Nudel recruits Lis1, dynactin, and dynein to the mother centriole and may transport MTs to be anchored to the subdistal appendages. (B) Nudel functions in both dynein-dependent and-independent centrosome protein assembly. It activates dynein for centripetal transport of PCM-1 (a). It also exhibits rapid turnover between cytosol and PCM (b) and facilitates centrosome targeting of pericentrin through direct interaction (c). -tubulin is promoted possibly through association with(Centrosomal targeting of pericentrin (d).

centriole in a dynamic, MT and dynein-independent manner. It is also critical for centrosomal targeting of dynein, dynactin, and Lis1, as well as MT anchoring at the mother centriole (Figure 2)[10]. Furthermore, we found that, although the assembly of pericentrin into the centrosome requires Nudel, dynein activity is dispensable (Figure 2). As Nudel interacted with pericentrin independent of dynein, we propose that its dynamic turnover at the centrosome facilitate centrosomal assembly of pericentrin, and possibly tubulin as well, independent of dynein (Figure 2)[10]. These results indicate conceptually that Nudel can recruit dynein/dynactin/Lis1 to certain target site, e. g., centrosome, and also has dynein-independent roles.

While working on Nudel, we also investigated possible functions of mitosin. It is found that depletion of mitosin by RNAi in M phase led to misaligned chromosomes, increased false MT-kinetochore attachment, premature chromosome decondensation before anaphase onset, and mitotic cell death[11]. Moreover, its depletion increased MT-dependent stripping of dynein/dynactin from the kinetochore, thus linking mitosin to dynein as well[11]. In addition, we identified another mitosin interactor, transcription factor ATF4, and showed that mitosin can serve as a negative regulator of ATF4, thus designating a role of nuclear mitosin in interphase[12].

To clarify whether mitosin affected retention of kinetochore dynein/dynactin through Nudel/NudE, we examined and found kinetochore localization of Nudel/NudE in M phase. Moreover, depletion of Nudel by RNAi or overexpression of NudelC36 resulted in severe mitotic block in prometaphase. While the poleward transport of kinetochore proteins was disrupted and spindle organizations became abnormal as expected, kinetochore localizations of dynactin, dynein, and Lis1 were progressively attenuated. Fur-

thermore, we found that Nudel was recruited to the kinetochore mostly by mitosin and only partly by dynein[13]. Similar to the case of mitosin[11], depleting Nudel increased MT-dependent stripping of dynein as well[13]. This not only confirmed Nudel as the mediator between mitosin and dynein, but also suggests that the MT-dependent stripping of dynein from the kinetochore is not due to its poleward pulling force because dynein is not supposed to be active in the absence of Nudel[13].

Kinetochore dynein has long been speculated to drive poleward chromosome movement in prometaphase/metaphase transition[14,15]. This issue, however, is still pending, since general inhibition of dynein before M phase inevitably disrupts spindle organization, whereas dynein inhibition in prometaphase via microinjection affects neither spindle nor chromosome congression[8,16]. We selectively eliminated kinetochore dynein/dynactin by RNAi-mediated depletion of ZW10, a protein essential for kinetochore localization of the motor complex (see Figure 3), and indeed visualized the disruption of poleward chromosome movement. Moreover, congression efficiency was also markedly reduced and cells frequently failed to achieve full chromosome alignment[17].

Therefore, mitosin appears to stabilize kinetochore dynein/dynactin by binding to Nudel to facilitate dynein-mediated poleward chromosome movement important for congression and full chromosome alignment (Figure 3)[13,17]. In the absence of mitosin, kinetochore dynein/dynactin tends to be edged out by MTs. Physiologically, this may facilitate the dynein-mediated poleward transport of kinetochore proteins (Figure 3).

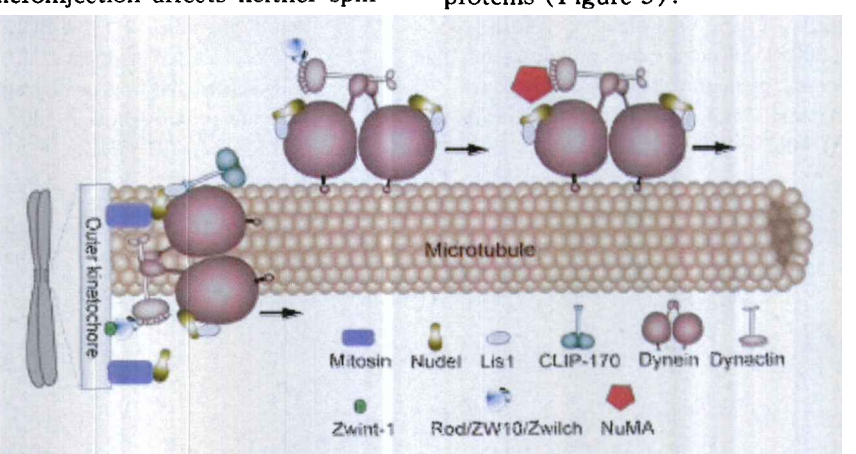

***Figure 3 Model for Nudel functions at the kinetochore*[13]**

Dynein/dynactin binds the kinetochore through the Rod/ZW10/Zwilch complex. Nudel is mainly recruited by mitosin, whereas a portion of it also binds dynein and Lis1 directly. Nudel on the one hand activates dynein-mediated poleward transport of outer kinetochore proteins to facilitate inactivation of the spindle checkpoint, and on the other hand, when interacting with mitosin as well, Nudel stabilizes dynein/dynactin against MT dependent stripping to facilitate the motor's force generation function for poleward chromosome movement and tension.

Despite these achievements, many questions still remain unanswered. For instances, why dynein requires Nudel and Lis1 for its activities in vivo? Does NudE function identically to Nudel? What are their dynein-independent functions? How are they related to human diseases? These questions can hopefully be answered in the future as research goes on.

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