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Ferritin forms dynamic oligomers to associate with microtubules in vivo: Implication for the role of microtubules in iron metabolism.

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Ferritin, a ubiquitously distributed iron storage protein, has been reported to interact with microtubules in vitro (Hasan et al., 2005, FEBS journal 272:822-831). Here, we demonstrate that ferritin binds with the microtubules in an oligomeric form and that the microtubule-bound ferritin contains more than two-fold amount of iron compared to the unbound ferritin fraction in vitro. Indirect immunofluorescence microscopy showed that a significant fraction of the ferritin molecules colocalized with the microtubules as oligomers, in a wide variety of cell lines. These findings are consistent with the immediate oligomerization of rhodamine-labeled ferritin, microinjected in living human hepatoma cells. Ferritin oligomers were dynamic in the cytoplasm, and an anti-microtubule drug significantly inhibited their intracellular movement. Treatment of cells with an iron donor, ferric ammonium citrate, remarkably increased the number of cells containing ferritin oligomers. On the other hand, when the cells, such as mouse neuroblastoma cells, were deprived of iron, ferritin oligomers were localized in the microtubule dense, neurite shafts, but were disappeared from the microtubule deficient neurite tips. These data indicate that the microtubules provide a scaffold for the cytoplasmic distribution and transport of the iron rich ferritin, and implicate the role of microtubules in iron metabolism.

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A microtubule-associated protein 4 specific five-repeat isoform inhibits kinesin-driven microtubule gliding

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Recently, we revealed that the isoforms of microtubule-associated protein (MAP) 4 alter the microtubule surface properties, and proposed a hypothesis that the physiological function of the isoforms, which differ from each other in the number of the repeat sequences, may be to regulate the interaction between microtubule and microtubule motors (Tokuraku et al., J. Biol. Chem.278, 29609-18, 2003). To verify the hypothesis, we examined whether the MAP4 isoforms affect motor activity of kinesin by in vitro gliding assay. The result revealed that the microtubules can glide on kinesin surface in the presence of the three-repeat or the four-repeat isoforms, while the microtubules stopped or paused in the presence of the five-repeat isoform. This result demonstrated that the five-repeat isoform alone inhibits motor activity of kinesin. To check whether the MAP4 isoforms interfere the binding of kinesin with microtubule, we analyzed the binding amount of kinesin to microtubule in the presence of the MAP4 isoforms by sedimentation assay. The result revealed that the binding of the kinesin to microtubules does not compete with all MAP4 isoforms. The absence of competition between the five-repeat isoform and kinesin, indicated that kinesin can bind to the five-repeat isoform-bound microtubules even though it is inhibited movement. The expression of the MAP4 isoforms depends on the tissue type and developmental stage. Our result suggests that MAP4 can regulate microtubule motor activity posttranscriptionally.

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Molecular basis of microtubule disassembly mechanism regulated by katanin p60.

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Katanin p60 (kp60), a type I AAA ATPases family member consisting of one conserved AAA domain, is known to regulate microtubule disassembly and severing. Most of type I and type II AAA ATPases contain unique N-terminal regions preceding to the AAA domains, that often recognize their specific substrate and/or adaptor.

We have identified a novel structural domain from the N-terminal regions of kp60. From this, we have succeeded to optimize the domain boundaries suited for structure determination by negative designs of putative coiled coil regions, using the program COILS1. Some variants of kp60 N-terminal domain (NTD) were analyzed by the 1H-15N HSQC and the analytical ultracentrifugation. The both results showed that the putative coiled coil region caused monomer-dimer equilibrium in solution. Based on this observation, we propose a novel but simple protocol for rational fine-tuning of the domain boundary2. Then, we have found tubulin binding activities within the isolated kp60 NTD. KP60 NTD also has indicated an inhibitory activity of microtubule polymerization. The solution structure of kp60 NTD has been determined using 15N, 13C-labeled kp60 NTD and is under refinement. KP60 NTD has comprised three anti-parallel α-helices, resembling to MIT domain of Vps4, another member of type I AAA ATPase. Similarity between not only the N-terminal structures but also the overall domain arrangement of kp60 and Vps4 will be discussed.

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Structural state transitions of G-actin studied by molecular dynamics simulation.

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Actin shows reversible transition between monomer (G) and fibrous (F) states known as G/F transformation, which is supposed to be regulated by the monomer structural state tightly coupled with the bound nucleotide state. X-ray and electron-microscope studies have suggested that the nucleotide state in G-actin is responsible for the structural states of the DNA-binding loop (D-loop) and of the cleft between the subunits 2 and 4. The X-ray structure indicated that the D-loop is in a random-coil state when ATP bound whereas it folds into an alpha-helix when ADP bound. Electron microscope showed that the cleft is in a closed state when ATP bound whereas it opens when ATP is hydrolyzed. In reality, the nucleotide-dependent cleft state is controversial because X-ray structure showed that the cleft remains closed even when ATP is hydrolyzed. To study these possible structural state transitions at atomic resolution, we conducted molecular dynamics simulation of G-actin in aqueous solution. We found that the D-loop is in equilibrium between helix and random-coil states as opposed to the X-ray structure: helix is not always stable in the ADP bound state and sometimes unfolds completely. The cleft was found to be closed both in the ATP and the ADP states, which is in agreement with the X-ray structure, while large fluctuation was observed in the nucleotide-free state. We further study the possible role of strictly conserved His73 in regulating the cleft structural state transition.