Structure and Function of Microtubule-depolymerizing Kin-I Kinesins

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Abstract Kin-I kinesins are one important class of microtubule regulatory proteins which can depolymerize microtubules in an ATP-dependent manner. They have critical functions in neuronal development, spindle assembly and chromosome segregation. Over the past ten years, a big amount of studies have been done to understand Kin-I kinesins. Here, several aspects of Kin-I kinesins are reviewed briefly, including the structure, mechanism of microtubule depolymerization and their physiological functions.

Key words Kin-I kinesins; microtubule; ATPase; depolymerization

Kinesins are one conserved class of microtubule-dependent motor proteins in all eukaryotic cells. They can use the energy of ATP hydrolysis to move along microtubules and transport different cargoes [1]. But Kin-I kinesins, namely kinesin-13, are an unusual class of kinesins. Structurally, unlike other kinesins, which have an N-terminal or C-terminal motor domain, Kin-I kinesins have a conserved motor domain located in the middle of the polypeptide. These Kin-I kinesins do not move along microtubules but regulate microtubule dynamics by depolymerizing microtubules in an ATP-dependent manner [2].

Microtubules are built from α/β tubulin heterodimers that assemble longitudinally to form protofilaments, 12–15 of which associate laterally to form microtubules [3,4]. As an important cytoskeleton, microtubules play essential roles in the maintenance of cytomorphology and intracellular transport [5]. On the other hand, microtubules are also related to cell division [6,7]. During mitosis and meiosis, the microtubule cytoskeleton is totally rearranged to form spindle, which drives the segregation of duplicated chromosomes [6,8]. The dynamics of microtubule is delicately regulated by a series of regulatory proteins including Kin-I kinesins [4,9].

Since the first kinesin was discovered in 1985 [10], more than 600 members of kinesin family from a variety of species have been reported so far [11,12]. These kinesins are mainly categorized into 14 families, but some kinesins that do not group to these 14 families are classifies as orphans [1,13]. Roughly, these kinesins can be classified into three types based on the position of the motor domain within the polypeptide, which is crucial for the function of kinesins. Most kinesins with the motor domain at the N-terminus [14] move toward the plus end of microtubule. By contrast, some kinesins have a C-terminally located motor domain and have microtubule minus-end-directed mobility [2]. However, some kinesins have a motor domain (Kin-Is) in the middle of the primary sequence [14] and function as microtubule depolymerase [2,8].

The first gene cloning of kinesin with an internal motor domain was obtained in 1992 [15]. Several years later it was established that Kin-I kinesins are microtubule-destabilizing enzymes [2]. Then it was found that "neck+motor" construct is the minimal functional domain of Kin-I kinesin [16]. So far, a lot of work has been done to understand the structure, function and the microtubule-depolymerizing mechanism of Kin-I kinesins.

1 Members of Kin-I Kinesin Family and Their Structures

Kin-I kinesins have three subfamilies: Kif2A, Kif2B and Kif2C [8]. The Kif2A subfamily includes *Xenopus* Kif2A (XKIF2), mouse mKif2A (MmKif2A) and *Homo sapiens*

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Kif2A (HsKif2A), etc. The Kif2B subfamily has quite few members. The members of Kif2C subfamily are relatively better studied, such as mitotic centromere-associated kinesin (MCAK), *Xenopus* KIF2C (XKCM1), mouse mKif2C (MmKif2C) and fish Kif2C (FKIF). Kin-I kinesins all have the conserved motor domain positioned in the middle of the sequence, and they mainly play important roles in cell division and neuron development.

Kin-I kinesins share a conserved motor domain with other conventional kinesins, but they also have specific structural elements (Fig. 1). The N-terminal globular domain is responsible for subcellular localization of Kin-I kinesins [17]. The neck region of 60 amino acids is a class-specific α-helix located at the N-terminus of the motor domain. This region has crucial function in microtubule depolymerization. The highly conserved motor domain (also called the catalytic core) positions in the middle of the primary sequence and has microtubule and ATP binding sites. The C-terminal domain contributes to the dimerization and the regulation of ATPase activity [6, 16]. Full-length Kin-I kinesins form dimers, but "neck + motor" constructs are monomers. Studies have suggested that the "neck + motor" part is essential for microtubule depolymerzation activity [16,18]. And the activity of the whole molecule is spatially controlled by the interplay between the N- and C-terminal domains during spindle assembly [19].

Fig. 1 The schematic diagram of Kin-I kinesins structure [4,17]

So far, 5 crystal structures of Kin-I kinesins have been determined. The crystal structures of pKinI (the *Plasmodium falciparum* motor domain, PDB-ID: 1RY6) [18] and the "neck+ motor" of MmKif2C (PDB-ID: 1V8J, 1V8K) [20] were determined in 2004. The crystal structures of human Kif2C motor domain (PDB-ID: 2HEH and 2GRY) were determined in 2006. The motor domain is composed of a central eight-stranded β-sheet with three α-helices on each face. Compared with other kinesin proteins, such as Kif1A (PDB-ID: 1I5S), the "neck +motor" constructs of Kin-I kinesins have specific conformation mainly at four regions (Fig. 2): Neck,

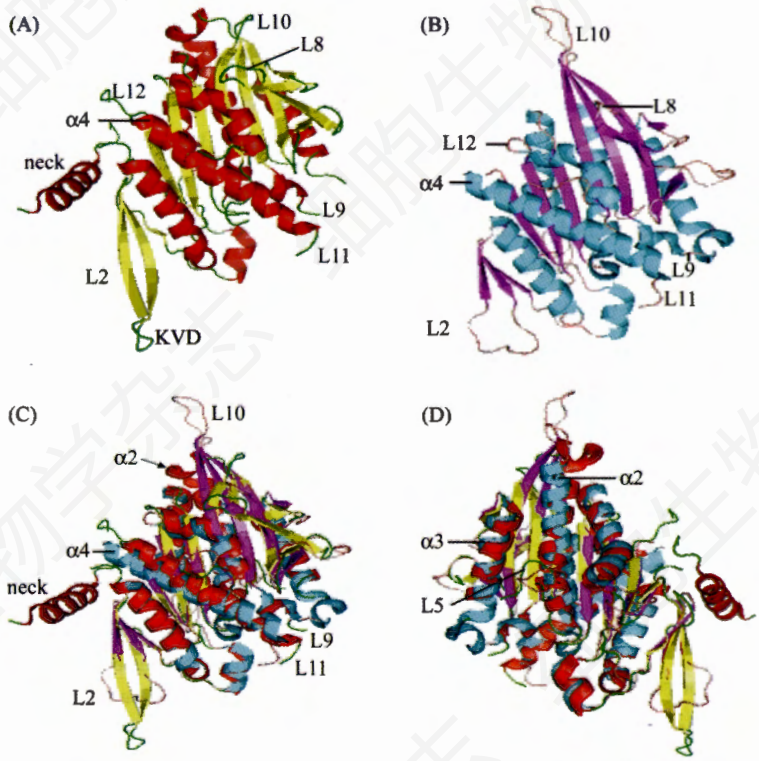

Fig. 2 Structures of Kif2C minimal functional domain, Kif1A motor domain and their superimposition

(A) Structure of KIF2C minimal functional domain (with Mg-ADP), PDB-ID: 1V8J; (B) Structure of Kif1A motor domain (with Mg-ADP), PDB-ID: 1I5S; (C) and (D) Views of the superimposition from two sides.

L2, switch I and switch II. Unlike the neck of Kin-Ns, the class-specific neck of Kin-I kinesins forms a long α-helix. When Kin-I kinesins bind to microtubule, this neck is supposed to extend vertically down to the groove between microtubule protofilaments and is essential for the microtubule depolymerizing activity [17]. In L2 there is a cluster of three residues (KVD), which is conserved in Kin-I kinesins. This loop is more than twice as long as L2 in other kinesins. Shipley *et al.* [18] demonstrated that this long loop is involved in specific interactions with microtubules. This KVD motif makes a rigid, finger-like protrusion extending out from the motor domain, and plays a critical role in microtubule depolymerization. Switch I, which comprises α2-L5, L8-α3-L9 (Fig. 2C, Fig. 2D), is responsible for ATP binding and the regulation of ATPase activity [20]. The α2 helix is interrupted by loop L5 in all kinesins. This loop is larger in Kin-Is than in Kif1A [21]. The kink of α2 and L5 dislocates α3, which leads to the dislocation of the preceding and following loops, L8 and L9 [20]. The displacement of switch I in Kin-I kinesins makes L9 unable to interact with ATP γ- phosphate. Switch II, which comprises L11-α4-L12, contributes to microtubule binding (Fig. 2C). The α4 helix of Kin-I kinesins is elongated and rotated compared with Kif1A, and the elongation of the α6 helix supports the α4 shift [18].

2 Mechanism of Microtubule Depolymerization by Kin-I Kinesins

Microtubule depolymerization by Kin-I kinesins occurs at both ends. When Kin-I kinesins bind to microtubule, the class-specific neck could insert vertically into the interprotofilament groove. It is generally believed that the neck might improve the efficiency of microtubule depolymerization by targeting the protein to microtubule ends [22]. The neckless MCAK mutants exhibited severely impaired microtubule depolymerization activity [22]. Moreover, Kin-I kinesins were shown not to move along the microtubules, but rather directly target to the microtubule ends [2] or move to the ends by rapid one-dimensional diffusion without using energy [23]. During microtubule depolymerization, Kin-I kinesins may make the end of protofilament curve. Indeed rings and spirals around microtubule have been observed. In this ring, the outer ring is formed by tubulin protofilament, the inner ring is formed by Kin-I motor [24,25].

Kin-I kinesins are ATPases, but ATP hydrolysis is not required to disassemble microtubules, because Kin-I kinesins bound to the nonhydrolyzable ATP analogue AMP-PNP induce microtubule protofilament peeling [2,24]. ATP hydrolysis seems necessary to release Kin-I kinesin from the complex with free tubulin heterodimer and complete a catalytic cycle [2]. It also appears that hydrolysis occurs before release of the tubulin-Kin-I complex from the microtubule, because MCAK has a higher ATPase activity in the presence of microtubules than free tubulin [23] and because AMPPNP-bound Kin-I kinesin forms rings from nonstabilized microtubules [24].

Two models have been proposed to illustrate the depolymerizing mechanism by Kin-I kinesins (Fig. 3). In the first model, Kin-I kinesins combined with ADP binds to the microtubule lattice. Then ADP exchanges with ATP. This ATP bound Kin-I kinesin targets to microtubule end by one dimensional diffusion and this binding to microtubule ends induces the conformational change of the protofilament. This step is critical in depolymerization. Subsequent ATP hydrolysis and phosphate release steps are required to dissociate the complex of Kin-I kinesin- tubulin dimer from the microtubule end and then dissociates Kin-I kinesin from tubulin dimer (Fig. 3A). In the second model, only the tubulin dimer is removed and Kin-I remains on the protofilament and continues to release the next tubulin dimer (Fig. 3B). This would explain the processivity of Kin-I kinesins [20].

3 Function of Kin-I Kinesins

Probably due to their ability to depolymerize microtubules, Kin-I kinesins have a variety of physiological functions. The Kin-I kinesins can participate in several events during mitosis including bipolar spindle assembly and chromosome movement, but they also have distinct functions [26,27].

The members of Kif2A subfamily are essential for both bipolar spindle assembly *in vivo* [28], and are involved in neuronal development [17,28]. Kif2A disassembles microtubules at spindle poles in association with poleward microtubule flux [29,30]. A recent study suggests

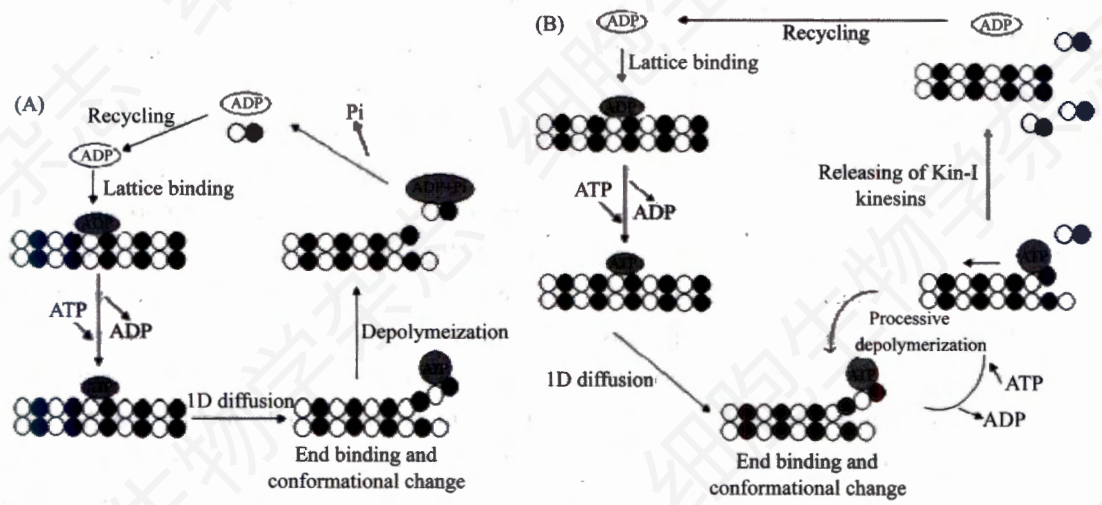

Fig. 3 Models of the microtubule depolymerization by Kin-I kinesins [2,20]

For simplicity, only one end of the microtubule is shown, although the depolymerization by Kin-I occurs at both ends. (A) During depolymerization, ADP bound Kin-I targets to the microtubule lattice and exchanges ATP. Then ATP bound Kin-I diffuses along the microtubule protofilament by 1D diffusion. Upon reaching the end of the microtubule, the curved conformation of the protofilament allows full contact with Kin-I. ATP hydrolysis takes place. The complex of tubulin and Kin-I is spontaneously released from the end. Hydrolysis of ATP on the complex releases Kin-I and the next cycle starts. (B) Alternatively, only the tubulin dimer is released and Kin-I remains on the protofilament, sliding back to release the next tubulin dimer processively. After removal a certain extent of tubulin dimers, Kin-I dissociates from the microtubule end and starts to recycle.

that XKIF2 regulates microtubule dynamics at the growth cone by depolymerizing microtubules and plays an important role in the suppression of collateral branch extension at the cell edge of post-mitotic neurons [11,31]. The cells lacking Kif2A form monopolar spindles in mitosis, and Kif2A-deficent neurons result in overextension of axons and branches.

The Kif2C subfamily members are involved in microtubule depolymerization in mitosis and meiosis. MCAK, which is the best-characterized member of this subfamily, mainly participate in bipolar spindle assembly and chromosome segregation. MCAK is enriched in spindle poles, kinetochores and centromeres during mitosis [26], in addition to associating with the tips of growing microtubules [32]. MCAK mutation or depletion of MCAK activity leads to improper spindle formation and to misaligned and lagging chromosomes during metaphase and anaphase [8,33].

By contrast, the function of Kif2B has not been well explored. A recent study demonstrated that Kif2B is essential for spindle assembly, chromosome segregation, and cytokinesis [27]. The cells lacking Kif2B can not assemble bipolar spindles but generally form monopolar or disorganized spindles. In Kif2B-deficient cells, the behavior and movement velocity of chromosomes are severely affected.

4 Conclusion and Perspectives

It has been more than ten years since the first gene clone of Kin-I kinesins was isolated [15]. Till now, significant progress has been made on many aspects of Kin-I kinesins. These studies revealed that Kin-I kinesins depolymerize microtubules and play important roles in cell division. But the details of the interaction between Kin-Is and tubulin are still unclear. Under physiological conditions, full-length Kin-I kinesins form dimer and this dimer would further increase the depolymerization activity, but whether dimerization of Kin-I kinesins *in vivo* is required for depolymerizing is unclear [20]. And the detailed molecular mechanism of ATP hydrolysis is still uncertain. Furthermore, the relationship between movement of other kinesins and the depolymerization mechanism by Kin-I kinesins remains unclear.

As Kin-I kinesins have critical functions in spindle

assembly, chromosome segregation and neuronal development, the research on them is particularly important. Firstly, the study on their enzymatic activity and biochemical characteristics can help to understand better their regulation and depolymerization mechanism. Secondly, the research in their mechanism has great significance and potential value in treating multitudinous diseases [34]. If specific small molecule inhibitor could be developed and applied, chromosomes of treated cells would be unable to separate normally, then effective mitosis would be perturbed [7,8], and the diseases would be healed. MCAK, which have been reported to be overexpressed in gastric cancer, would be a potential target for anticancer drug and cancer-specific immunotherapy [35]. So, further studies on Kin-I kinesins at different levels, from structure to biological function to clinic treatment, would be of great interest.

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微管解聚型驱动蛋白的结构与功能

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摘要 Kin-I 驱动蛋白(Kin-I kinesins)是一类重要的微管调节蛋白, 具有依赖 ATP 的微管解聚活性。这类驱动蛋白在神经元的发育、纺锤体的组装和染色体的分离过程中起着重要的作用。自被发现以来的十几年里, 人们对 Kin-I 驱动蛋白做了大量的研究工作。现对 Kin-I 驱动蛋白的结构、微管解聚活性及生理功能等方面进行简要综述。

关键词 Kin-I 驱动蛋白; 微管; ATPase; 解聚

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