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Kinesin-8s hang on by a tail

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Accurate segregation of genetic material into two daughter cells is essential for organism reproduction, development, and survival. The cell assembles a macromolecular structure called the mitotic spindle, which is composed of dynamic microtubules (MTs) and many associated proteins that assemble the spindle and drive the segregation of the chromosomes. Members of the kinesin superfamily of MT associated proteins use the energy of ATP hydrolysis to help organize the spindle, to transport cargo within the spindle, and to regulate spindle MT dynamics. The Kinesin-8 and Kinesin-13 families are involved in controlling mitotic spindle morphology, spindle positioning, and chromosome movement. While both kinesin families are MT destabilizing enzymes, it is unclear whether their mechanisms of MT destabilization are mechanistically similar or how they act to destabilize MTs. Recently, three groups identified an additional MT binding domain within the tail of Kinesin-8s that is essential for their roles in regulating MT dynamics and chromosome positioning.

Keywords: kinesin-8, microtubule dynamics, spindle assembly, mitosis, depolymerase

Abbreviations: ATP, adenosine tri-phosphate; EB1, end binding protein 1; Kif, kinesin family member; Kip3, kinesin related protein 3; Klp5/6, kinesin-like protein 5/6; MAP, microtubule-associated protein; MCAK, mitotic centromere-associated kinesin; MT, microtubule; Ran-GTP, Ran guanosine tri-phosphate

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Properly regulated microtubule (MT) dynamics are critical for assembly of the mitotic spindle and for the precise alignment and segregation of chromosomes into two daughter cells.[1] Spindle components, such as MT associated proteins (MAPs) and motor proteins, have been identified as cytoplasmic factors that can regulate the dynamics of MTs through either stabilization or destabilization mechanisms. Two important classes of kinesins, the Kinesin-13 and Kinesin-8 families, play essential roles in destabilizing MTs and highlight the diverse mechanisms that cells use to provide temporal and spatial control over the MT cytoskeleton. A series of recent papers have provided significant new insight into how these different kinesins control MT dynamics.[2-4]

Members of the Kinesin-13 family control the dynamics of both astral and kinetochore MTs, are required for bipolar spindle formation, for chromosome alignment, and to prevent or correct errors in the attachment of chromosomes to the spindle.[5,6] Biochemically, the Kinesin-13 family members do not walk along MTs, but rather they are potent MT depolymerases that enrich on MT ends and induce a conformational change at the protofilament end that leads to MT depolymerization.[7,8] In contrast, Kinesin-8 proteins are plus-end directed motors and plus-end specific MT destabilizing enzymes.[9-12] The functions of Kinesin-8s are diverse and include proposed roles in spindle assembly,[13] spindle positioning,[14,15] spindle length control,[16] chromosome positioning,[10,17-19] and anaphase chromosome segregation.[20,21] Despite all of these functional studies, the mechanisms by which Kinesin-8s control MT dynamics are not understood.

The most significant mechanistic insights into the Kinesin-8 family have come from studies on the budding yeast Kinesin-8 Kip3, which showed that these motors are MT plus-end directed motors that have a preference for destabilizing long MTs as opposed to shorter MTs.[9,11,12] From these studies, it was proposed that the MT length dependent destabilization induced by Kinesin-8s is proportional to the number of Kinesin-8 motors that are bound to the MT. In this model, motors are able to randomly bind to a MT, where the number of motors bound to a MT at a

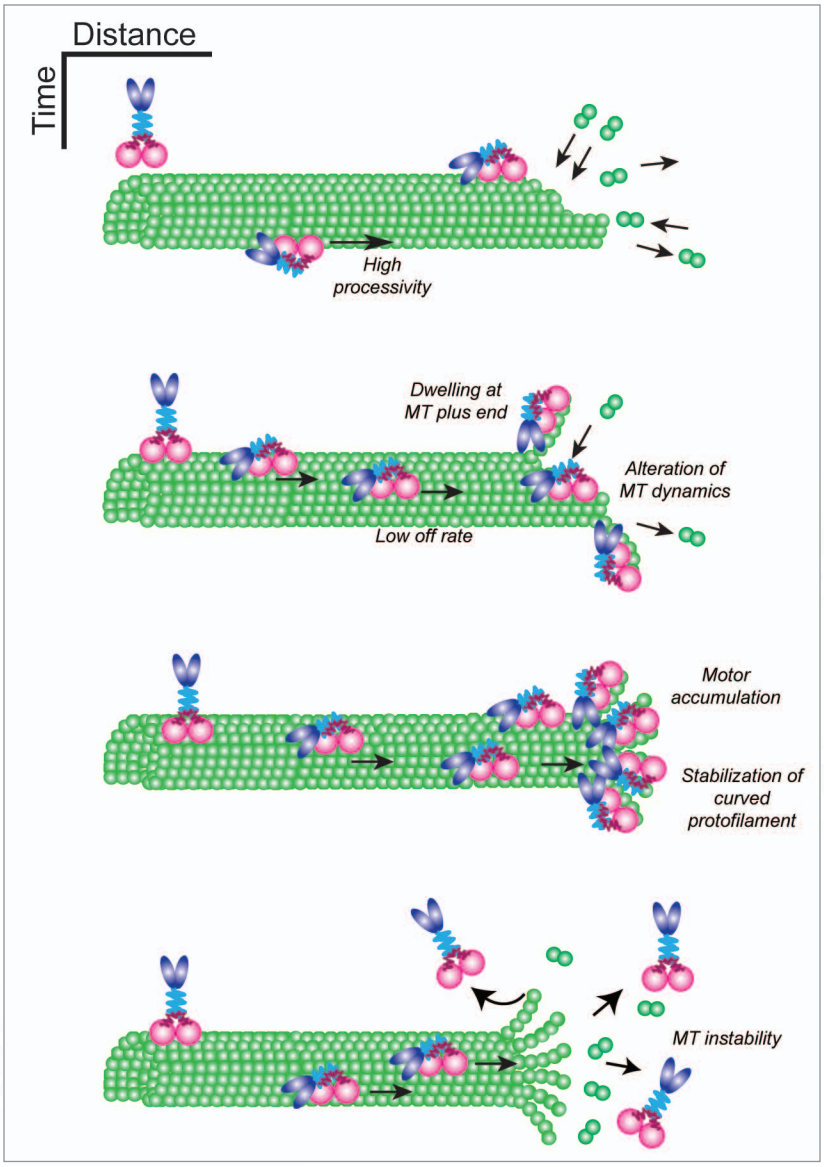

Figure 1. Schematic of Kinesin-8 motors binding and walking along a MT toward the plus end. Over time, Kinesin-8 motors accumulate and dwell at the plus end of the MT. Once properly localized, Kinesin-8 motors stabilize a curved protofilament conformation until enough motors have accumulated, and the MT undergoes a catastrophe. It is possible that as motors accumulate and stabilize the curved conformation the net effect would be to act as a capping protein wherein tubulin subunits are neither gained nor lost from the MT end.

given time is proportional to the length of the MT. Kinesin-8 proteins with high processivity are able to reach and accumulate at the MT plus end where a concentration gradient is formed, resulting in the depolymerization of long MTs.[11] This concentration gradient is also proposed to be important in how Kinesin-8s act cooperatively in destabilizing MTs.[12]

In contrast to the situation in budding yeast, results in other systems are contradictory regarding the mechanisms by which Kinesin-8 proteins contribute to MT destabilization. In fission yeast, Klp5/6 are proposed to be MT destabilizing enzymes in vivo,[20,22] but they do not actively depolymerize MTs in vitro.[23] In mammalian cells, some studies showed that the Kinesin-8 Kif18A can directly depolymerize MTs,[10] while other reports suggest that it is a MT plus-end capping protein.[24] Studies on all Kinesin-8s agree that these motors are highly processive (they move long distances on the MT without dissociating) and concentrate in a gradient dependent manner at the plus ends of MTs where the motors cooperate to induce MT destabilization.[12,24] Cryo-EM studies of the motor domain bound to MTs showed that Kinesin-8 motors can bind adjacently along protofilaments to stabilize a curved structure,[25] suggesting a mechanism in which multiple Kinesin-8s accumulate at the plus end of a MT to induce depolymerization by stabilizing the bent structure of the MT. In addition, three groups recently showed that the tail domains of Kip3 and Kif18A possess an ATP-independent MT binding domain that binds to MTs in vivo and in vitro and is important for proper localization and function of the molecule.[2-4] These studies are helping us understand what features of Kinesin-8 proteins are conserved and what features are different to allow them to carry out diverse functions on controlling the MT array.

Longer mitotic spindles observed in Kip3 deletion strains[21] suggest that Kip3 affects parameters of dynamic instability, where deletion of Kip3 resulted in a decrease in MT catastrophe frequency and a reduction of time MTs remained in a paused state (MTs are neither growing nor shortening).[9] This result suggests that Kip3 has the ability to destabilize MTs by increasing the frequency of catastrophes as well as to stabilize MTs by preventing the addition or loss of MT polymer. Su et al.[3] were interested in understanding how the destabilizing and stabilizing effects of the yeast Kip3 were coordinated. The authors generated a yeast strain that expressed only a tailless version of Kip3 and found that these cells exhibited resistance to the MT destabilizing drug benomyl, similar to Kip3 knockout cells, suggesting that without the tail domain MTs are more stable due to ineffective Kip3. Using purified versions of Kip3 in single molecule tracking experiments, the authors demonstrated that the tail domain of Kip3 is important for processivity, increased MT plus-end dwell time, and MT depolymerization. From their studies they proposed a model whereby Kip3 utilizes its highly processive motor domain to translocate itself to the MT plus end. Plus-end accumulation is achieved by the strong association with the MT end mediated by the

Kip3 tail, which allows Kip3 to cooperatively induce MT depolymerization. The tail also has a second role in mediating MT stabilization along the length of the MT, which will in turn allow more Kip3 to accumulate at the MT plus-ends and destabilize the MTs.

Studies on the mammalian ortholog Kif18A, also demonstrated that it utilizes its tail to increase motor processivity and MT plus-end dwell time.[2] In addition, human cells expressing a tailless Kif18A construct are unable to localize this tailless Kif18A properly to the plus ends of MTs, decrease the length of the spindle, or suppress chromosome oscillations in comparison to overexpressed full-length Kif18A.[2,4] These studies suggest that the tail domain of Kinesin-8s is required to tether the molecule to the MT plus ends where the motors are able to accumulate in order to promote MT destabilization. Consistent with this hypothesis, mathematical modeling using dynamic MTs suggests that high processivity and an increased dwell time at the MT plus ends are required for dampening of MT dynamics, resulting in a population of short MTs compared with conditions without an added motor.[4] Together these studies suggest that unlike Kinesin-13s that promote MT destabilization by increasing the frequency of catastrophe (transition from MT growth to shrinkage), Kinesin-8 motors likely act in a cooperative fashion such that sufficient amounts of protein need to accumulate at the MT plus end in order to promote MT destabilization (**Fig. 1**).

Based on what is known about Kinesin-8s, one might speculate whether Kinesin-8 proteins are truly depolymerases or perhaps just motor proteins that can alter multiple MT dynamics parameters in order to adjust the length of the MT. Unlike Kinesin-13s that bind to the end of the MT and immediately induce catastrophe,[7] accumulation of Kinesin-8 proteins is necessary to promote MT destabilization.[12] Multiple studies have shown that Kinesin-8s in different organisms accumulate at the plus end of the MT where the motor dwells for lengthy periods of time.[12,24] Even though a Kinesin-8 molecule dwells at the plus end of a protofilament, it is not actively depolymerizing the MT, suggesting that multiple motors are needed to be positioned on adjacent protofilaments in order to destabilize a MT.[12] In addition, Kip3 is a less robust depolymerase compared with MCAK, which depolymerizes MTs at a rate almost two-fold faster.[11,26] Kip3 has a maximum depolymerization rate for longer MTs, and as the MT becomes shorter the rate of depolymerization decreases; whereas, the depolymerization rate for MCAK does not change in a length dependent manner.[11] These observations suggest that instead of robust MT depolymerases that induce MT catastrophes like Kinesin-13s, Kinesin-8s are motors that "tune" MTs to a specific length in order to destabilize MTs. This idea is consistent with the predictions of computational modeling studies, which suggest that while the net effect of adding either Kinesin-13s or Kinesin-8s to a population of dynamic MTs leads to shorter average MT lengths, the amount of Kinesin-8 proteins required for the effect exceeds that of the Kinesin-13 proteins.[4]

It is also interesting to speculate how the tails of kinesins evolved their unique roles. Different kinesin families utilize their tail domains to perform multiple functions on the MT cytoskeleton. For most kinesins, the tail domain has been viewed as the cargo-binding domain;[27] however, depending on the kinesin, the tail sometimes perform regulatory roles as well. In conventional kinesin (Kinesin-1), the tail domain interacts with the motor domain to keep the motor in a closed conformation until it interacts with cargo.[28] For the Kinesin-14 proteins, there is a second MT binding site in the tail domain,[29] which allows Kinesin-14s to cross-link MTs.[30,31] This tail-binding site can also be utilized as a regulatory domain when bound by importin α/β to prevent MT cross-linking in areas of low Ran-GTP.[32] Interestingly, while the Kinesin-8 proteins contain a tail domain, not all Kinesin-8 proteins use that tail as a second MT binding domain. The human paralog of Kif18A, Kif18B, contains a binding site for the MT plus-end binding protein EB1 within the tail domain, suggesting that Kif18B uses an indirect mechanism to target to MT ends. Association of Kif18B with EB1 is required for its localization and for its ability to destabilize astral MT plus ends.[33,34] One interesting question is why two different Kinesin-8 proteins would use distinct mechanisms to target to MT plus ends. One hypothesis is that Kif18A needs MTs with sufficient stability, such as the bundles of MTs that compose the kinetochore-fiber, in order for the motor to accumulate at the plus ends before that MT would undergo depolymerization through dynamic instability. In contrast, Kif18B acts on the plus ends of the more dynamic astral MTs, so maybe EB1 somehow marks those MTs as being more stable, and thus allows for Kif18B to accumulate sufficiently to regulate their dynamics. Another interesting question is why Kip3 is the only Kinesin-8 that has MT depolymerizing activity in vitro. Because budding yeast do not contain Kinesin-13 motors, Kip3 may be an enzyme that possesses the ability to depolymerize MTs at the onset of mitosis and then modulate MT dynamics through its ability to also stabilize MTs.

While these studies answer important questions about the conservation of mechanism between the Kinesin-8 proteins, they leave several questions unanswered. First, it is unclear what parameters of MT dynamics are altered by Kif18A and other Kinesin-8 proteins during mitosis and whether depletion of Kif18A will affect multiple parameters of dynamic instability similarly to knockout of Kip3. From a mechanistic standpoint, it will be interesting to determine how stabilizing MTs in a curved conformation by the Kinesin-8 motor domain induces MT instability. In addition, it will be important to reconstitute dynamic MTs with the Kinesin-8 proteins to see what aspects of dynamic instability are regulated. Finally, a major question is why do higher eukaryotes have both Kinesin-13s and Kinesin-8s (7 proteins in all) to regulate MT dynamics when yeast can get by with just Kip3. Dissecting the unique aspects of these motors will be informative for understanding how the dynamic MT cytoskeleton is involved in diverse cellular processes.

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