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Mechanisms for regulation of plant kinesins

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Abstract

Throughout the eukaryotic world, kinesins serve as molecular motors for the directional transport of cellular cargo along microtubule tracks. Plants contain a large number of kinesins that have conserved as well as specialized functions. These functions depend on mechanisms that regulate when, where and what kinesins transport. In this review, we highlight recent studies that have revealed conserved modes of regulation between plant kinesins and their non-photosynthetic counterparts. These findings lay the groundwork for understanding how plant kinesins are differentially engaged in various cellular processes that underlie plant growth and development.

Highlights

- Kinesins are motor proteins that transport cargos along microtubule tracks
- Plants contain a large number of kinesins, which have important functions during the cell cycle
- Kinesin activity is regulated by protein-protein interactions and covalent modifications to ensure that cargos are delivered at the right time to the right place.
- Better understanding of these regulatory mechanisms is essential to learn how kinesins mediate vital cellular processes.

Introduction

Directional transport of cellular material is essential for the form and function of cells. This is largely driven by molecular motors, which use the chemical energy from ATP hydrolysis as fuel to move on cytoskeletal filaments [1-2]. The kinesin and dynein molecular motors move along microtubules, whereas myosins move on actin filaments. In plants, most of the long-distance intracellular transport during interphase is mediated by the actin-myosin cytoskeletal system [3]. However, kinesins are emerging as important players during interphase, both for cortical microtubule organization and for cell wall assembly [4]. In addition, kinesins have essential roles during mitosis, orchestrating the assembly and function of the preprophase band, spindle apparatus and phragmoplast [4-5].

Kinesins primarily work as plus-end-directed motors (Box 1), whereas dyneins are responsible for the bulk of minus-end-directed transport in animal cells. Genome sequencing has revealed that land plants lack dynein and encode for a large number of kinesins, many of which are predicted to be minus-end-directed motors that might compensate for the lack of dynein [6-8]. Several recent reviews have summarized the identity, phylogenetic relationships and functions of plant kinesins [4,9-12]. Here, we focus on mechanisms that regulate kinesin activity, which collectively ensure that kinesins function at the right time and place in a cell.

- **Box 1: Kinesin structure and motor properties**

The basic functional unit of most kinesins is a dimer of two kinesin heavy chains (KHCs) that consist of motor, neck-linker, stalk and tail domains (Figure 1). The globular motor domain is the catalytic core with both ATP- and microtubule-binding sites. The neck-linker acts as a lever arm that changes conformation upon ATP binding in the leading motor domain to thrust the lagging motor domain forward. The amino-acid sequence of the neck-linker also specifies the directionality of kinesin movement. The extended stalk domain is primarily composed of a coiled-coil and leads to the tail domain that mediates cargo binding. The tail domain is highly divergent between different kinesins and is thought to at least partly underlie the ability of distinct kinesins to transport different cargos. Apart from these canonical domains, some plant KHCs also contain unique domains (e.g. calponin homology, armadillo repeat, myosin tail homology and band 4.1 domains) [6,7] that might be important for plant-specific functions. While most kinesins are homodimers, the kinesin-1 subfamily is a heterotetramer of two KHCs and two kinesin light chains (KLCs). The KLCs associate with the coiled-coil of the stalk domain and contribute to cargo recruitment and regulation of motor activity.

Kinesins move along microtubules in a directional manner. In general, kinesins with an N-terminal motor domain move towards the microtubule plus-end (the β-tubulin end), whereas kinesins with a C-terminal motor domain move towards the microtubule minus-end (the α-tubulin end). In contrast, kinesins with a centrally located motor domain generally are not motile and instead depolymerize microtubules from the ends. In most motile kinesins, the microtubule binding and unbinding activities of the two motor domains are coordinated, allowing for long-distance or processive movement of kinesins along microtubule tracks.

Regulation of kinesin function

For motile kinesins, the basic functional steps are: binding to appropriate cargo and/or recruitment to the correct subcellular location, activation of motility and release of cargo at the correct destination. Together, these steps ensure that different cargos are transported to their proper cellular locations. Work in animal and yeast systems has shown that these steps involve different domains of kinesins: the motor and neck-linker domains determine the speed, distance and directionality of transport whereas cargo binding typically occurs via the tail domain [2,13]. However, the tail domain can also interact with the motor domain in the absence of cargo to regulate motor activity. In addition, other non-motor regulatory domains have been identified that either positively or negatively regulate motor activity depending on protein-protein interactions, phosphorylation, Ca^{2+}-calmodulin activity and proteolysis. Together, these regulatory mechanisms directly act on kinesins to coordinate the complex process of kinesin-cargo coupling, transport and cargo unloading [1,13].

Recently, several studies have begun to uncover mechanisms for regulating kinesin activity in plants. Comparison of these mechanisms between plants and animals reveals many conserved regulatory features as well as new ones that might be unique to plants. These are discussed below based on their mode of action.

- **Autoinhibition and KLC-mediated regulation**

Kinesin motor activity is autoinhibited in the absence of cargo to prevent futile consumption of ATP and overcrowding of microtubule tracks that might lead to traffic jams. Autoinhibition happens primarily by two mechanisms: 1) kinesin adopts a folded conformation that enables the tail domain or certain internal segments to interact with the motor domain and block microtubule binding and ATPase activity [14-20]; and 2) KLCs inhibit the processive motility of kinesin by preventing coordinated movement of the two motor domains [14,21]. In metazoan kinesins, a hinge region in the stalk domain allows the molecule to fold for autoinhibition. Release of autoinhibition is accomplished by cargo binding to the tail domain and/or to KLCs [13]. Alternatively, motor activation can also occur by phosphorylation of specific residues in the tail domain [22-23].

Based on sequence and structural homology with the mammalian KLC subunits, *Arabidopsis* is thought to encode for three KLC-related (KLCR) proteins [24••]. The function of these proteins is not clear since plants do not have a bona fide kinesin-1, of which KLC is normally a subunit. Recently, KLCR1 was found to directly interact with a plant-specific calmodulin-binding protein called IQD1 (IQ67 Domain1) [24••]. IQD1 localizes to interphase cortical microtubules when transiently expressed in tobacco leaves and is necessary for KLCR1 localization to microtubules in this heterologous system. Based on these data, IQD1 has been proposed to work as a scaffolding protein that recruits kinesin motors via KLCR1 to particular cellular cargo (Figure 2A). However, whether KLCR1 binds to a kinesin motor protein and regulates its motility and cargo loading remains to be determined. IQD1 can bind to artificial single-stranded nucleic acids *in vitro*, raising the possibility that it might serve as an adaptor for kinesin-mediated transport of ribonucleoprotein complexes, similar to kinesin-1-mediated transport of messenger RNA is mouse neurons [25-27]. However, this activity needs to be experimentally demonstrated in plant cells.

- **Regulation by phosphorylation**

Kinesins are known phosphoproteins and phosphorylation can regulate both kinesin motor activity and cargo loading and unloading [1,13]. In mammals, phosphorylation of kinesin-1 motors by the JUN amino-terminal kinase (JNK) weakens microtubule affinity, thus reducing motor activity [28]. Conversely, for kinesin-5 and kinesin-7 members, phosphorylation in the inhibitory C-terminal domain has been shown to unfold these motors, thus stimulating their motility [22-23]. In *Arabidopsis*, the kinesin-14 family members, Kinesin CDKA-Associated 1 and 2 (KCA1 and KCA2) have been shown to interact with the cell cycle-dependent protein kinase CDKA;1 both *in vitro* and *in vivo* [29]. These kinesins are implicated in marking the cell division site defined by the preprophase band [30]. Phosphomimetic mutations at two consensus CDKA;1 phosphorylation sites in the tail domain of the KCA kinesins prevent interaction with wild-type C-terminal tail fragments of KCA1 and KCA2 in directed yeast two-hybrid assays [29]. These data suggest that phosphorylation of the tail domain of KCA1 and KCA2 by CDKA;1 might regulate motor activity by affecting their dimerization and/or conformation. However, these kinesins do not show detectable microtubule-binding activity in sedimentation assays, probably due to lack of canonical microtubule-binding residues in their motor domains [31•]. Therefore, it is possible that KCA1 and KCA2 are not motile kinesins and function in other ways. Recently, KCA1 and KCA2 were found to be important for light- and actin-dependent chloroplast movement and it was proposed that this function might depend on binding to F-actin through their C-terminal domain [31•]. It will be informative to determine whether the phosphomimetic versions of KCA1 and KCA2 are significantly affected in their ability to bind to F-actin and support light-dependent chloroplast movement. These data might also inform our understanding of KCA1 and KCA2 function during cell division since the KCA1/2-depleted cortical zone that is proposed to mark the cell division site [30] mirrors the actin-depleted zone that is thought to perform a similar function [32].

In plants, the turnover of phragmoplast microtubules during cytokinesis is controlled by a mitogen-activated protein kinase (MAPK) cascade. Initiation of this MAPK cascade depends on kinesin-like proteins called NACK1 and NACK2 (NPK1-activating kinesin-like protein 1 and 2), which directly interact with and activate the MAP kinase kinase kinase protein, NPK1 (nucleus- and phragmoplast-localized protein kinase 1) [33]. The interaction of NACK1/NACK2 with NPK1 in turn is regulated by cyclin-dependent kinases (CDKs). Both NACK1 and NPK1 are phosphorylated by CDKs at the onset of mitosis, which inhibits their interaction and blocks progression into cytokinesis [34••]. During late metaphase, when CDK activity becomes low, NACK1 and NPK1 become dephosphorylated and interact, thus mediating the transition from metaphase to cytokinesis (Figure 2B). An elegant test of this model was employed using a phosphomimetic mutant version of NACK1, which failed to rescue the cytokinesis defect of *Arabidopsis atnack1-1* mutants [34••].

CDK-mediated regulation is likely to be common for kinesins involved in mitosis [35]. The rice BC12 kinesin contains conserved CDKA phosphorylation sites in its tail domain and interacts with CDKA;3 in yeast two-hybrid assays [36]. In addition, the *bc12* mutant shows delayed cell cycle progression. These data suggest that CDKA might regulate BC12 activity during the cell cycle, but whether BC12 is phosphorylated and whether this affects its activity remains to be determined. Other mitotic plant kinesins such as AtKINUa (At1g12430), AtPOKs (At3g17360 and At3g19050), AtKinG (At1g63640) also contain putative CDKA phosphorylation sites, but their role remains unknown [12,35,37•]. Phosphomimetic and phosphodefective mutations should shed light on whether and how these sites regulate kinesin function.

- **Regulation by the Ca^{2+}/Calmodulin pathway**

Ca^{2+}-calmodulin (Ca^{2+}/CaM) mediated regulation has been mostly attributed to cargo binding and dissociation in mammalian cells [13]. In plants, our knowledge of Ca^{2+}/CaM-dependent regulation of kinesin activity comes primarily from a plant-specific, minus-end-directed kinesin called KCBP (Kinesin-like calmodulin-binding protein) [38,39]. KCBP has a CaM-binding domain following its C-terminal motor domain and biochemical studies have shown that *Arabidopsis* KCBP can bind at least three different CaMs [40]. KCBP binds microtubules in the absence of Ca^{2+}/CaM, whereas in the presence of Ca^{2+}/CaM the microtubule binding affinity and ATPase activity of KCBP is reduced [41,42]. Another Ca^{2+}-binding protein, KIC (KCBP-interacting Ca^{2+}-binding protein), was also found to regulate KCBP in a similar manner [43]. The crystal structure of KCBP has provided insight into the mechanism of Ca^{2+}/CaM and Ca^{2+}/KIC mediated regulation [44-46]. The Ca^{2+}/CaM-binding helix of KCBP is linked to the motor domain by a polypeptide segment that resembles the motor neck domain. When Ca^{2+} levels are low, this neck mimic binds to the motor domain and positions the CaM-binding helix such that it is available to bind to CaM. In the presence of Ca^{2+}, activated CaM binds this exposed helix and greatly decreases microtubule binding affinity by sterically blocking residues important for motor-microtubule contact and by enabling the negatively charged extreme C-terminus of KCBP to associate with the microtubule-binding interface of the motor domain (Figure 2C). Ca^{2+}/KIC-based regulation operates through a slightly different mechanism. Binding of active KIC to the exposed regulatory helix of KCBP causes the neck mimic to interact with KIC and traps the motor domain in the ADP state, which has low affinity for microtubules.

An analogous pathway for Ca^{2+}/CaM-mediated deactivation of kinesin-1 has been described in *Drosophila* [47]. In *Drosophila* neurons, kinesin-1 is important for distribution of mitochondria along the axon length. Kinesin-1 interacts with a mitochondrial membrane protein Miro through an adaptor protein called Milton. At resting Ca^{2+} levels, kinesin-1 transports mitochondria along axonal microtubules. At elevated Ca^{2+} levels, Ca^{2+}-bound Miro directly interacts with the kinesin-1 motor domain thereby dissociating kinesin-1 from microtubules and preventing mitochondrial transport [47]. It will be interesting to determine whether Ca^{2+}/CaM and Ca^{2+}/KIC locally regulate KCBP activity to control cargo transport in cells. In addition, it is not clear why multiple Ca^{2+}-based regulatory mechanisms exist for KCBP. One possibility is that these are differentially engaged in different cell types or at different stages of the cell cycle to control microtubule bundling and/or sliding activity of KCBP, which might be important for the assembly and/or stabilization of microtubule arrays [4].

- **Regulation by the proteasome pathway**

Targeted degradation of proteins by ubiquitination and proteasome-mediated proteolysis is important for proper progression through the cell cycle. As kinesins are major players during cell division, their activity might be regulated by the proteasome system [35]. The first example of proteasome-based kinesin regulation in plants comes from an ungrouped kinesin called AtKINUa/ARK3 (Figure 2D). AtKINUa contains a destruction box (D-box) motif as part of its motor domain [37•,48]. The D-box is a recognition motif for the ubiquitination machinery during mitosis [49], which might lead to degradation of AtKINUa in a cell-cycle-stage dependent manner. Consistent with this expectation, AtKINUa protein accumulates in the preprophase band and its levels decline sharply after nuclear envelope breakdown in prometaphase. Importantly, mutation of the D-box motif inhibited the loss of AtKINUa during mitosis, indicating that

targeted degradation is likely to be important for regulating AtKINUa function [37•]. Recently, the KCBP homologue of the microalgae *Dunaliella salina* was reported to interact with a 26S proteasome subunit Rpn8 in yeast two-hybrid assays [50]. This KCBP was found to be polyubiquitinated and undergo degradation by the proteasome system. Therefore, proteasome-mediated kinesin regulation is likely to be a widely used mechanism in the plant kingdom.

Conclusions

While genome sequencing has greatly facilitated kinesin discovery in plants, our understanding of the function and regulation of plant kinesins is restricted to just a small subset. Nonetheless, published data show that multiple regulatory mechanisms exist to control kinesin activity in plants, similar to the situation in animal cells. While we have discussed these mechanisms separately, it is important to note that they may act in concert to fine-tune motor activity. For example, IQD1 activity is regulated by Ca^{2+}/CaM, which in turn could control KLCR1-based kinesin activation and transport. Understanding the pathways that engage the different regulatory mechanisms and their functional consequences on kinesin activity are major challenges for future research.

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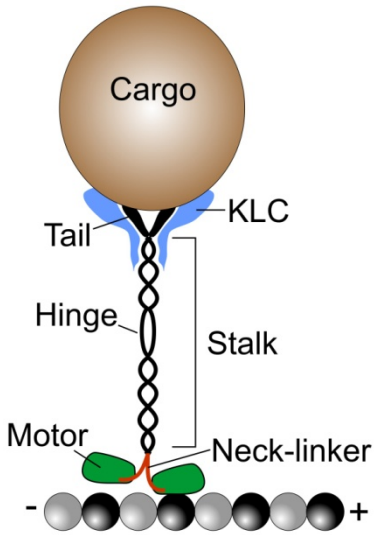

Figure 1: Schematic representation of kinesin structure. A heterotetrameric kinesin is depicted to illustrate the various functional domains of kinesins. For simplicity, only a single protofilament of the microtubule is shown here.

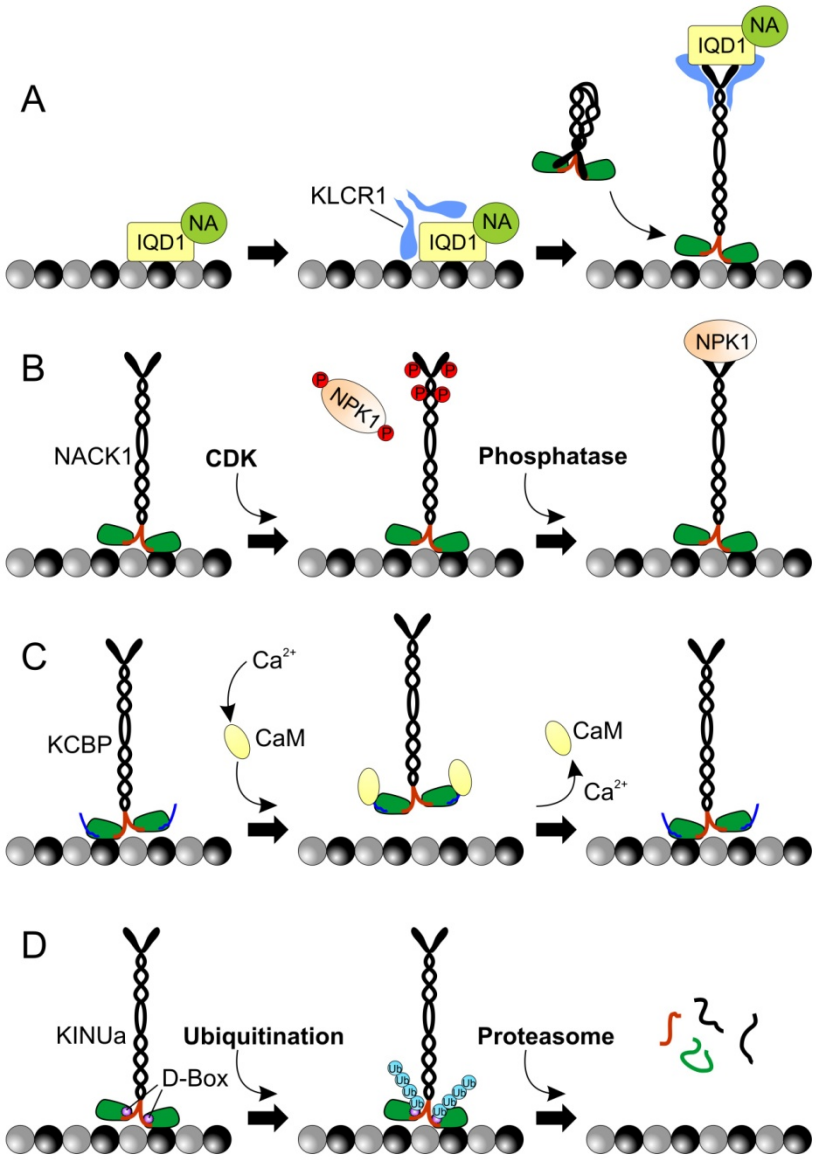

Figure 2. Mechanisms for regulating plant kinesins. (A) The scaffold protein IQD1 binds to interphase cortical microtubules and possibly to nucleic acids (NA). IQD1 also binds to KLCR1 (kinesin light chain related 1), which is proposed to recruit kinesin motors for transport. (B) During prophase, phosphorylation of the kinesin NACK1 and its binding partner NPK1 by CDK1 inhibits their interaction. Dephosphorylation of these proteins during late metaphase allows them to interact, initiating a signaling cascade that is necessary for phragmoplast assembly and cytokinesis. (C) The KCBP kinesin has a CaM-binding domain following its C-terminal motor domain and can bind at least three different CaMs. Increased Ca^{2+} levels activate CaMs, which then directly bind to the CaM binding domain thereby deactivating the motor by reducing its affinity for microtubules. (D) The AtKINUa kinesin possesses a destruction-box (D-box) motif in its motor domain. Based on the disappearance of this kinesin upon nuclear envelope breakdown, it is proposed that the D-box acts as a recognition motif for polyubiquitination and subsequent proteasome-mediated degradation at prometaphase.